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INVESTOR IN PEOPLE

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NP10 8QQ

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Dated 24 March 2003

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Request for grant of a patent

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The Patent Office
Cardiff Road
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1. Your reference — SCH/HG/P33025

2. Patent application number
(The Patent Office will fill in his part) — 0207283.3

28MAR02 E707169-1 C69803
P01/7700 0.00-0207283.3

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Glaxo Group Limited
Glaxo Wellcome House, Berkeley Avenue,
Greenford, Middlesex UB6 0NN, Great Britain

Patents ADP number *(if you know it)* 473587003

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom
see continuation sheet for further applicant(s)

4. Title of the invention — Novel Compounds

5. Name of your agent *(if you have one)*

Corporate Intellectual Property

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

GlaxoSmithKline
Corporate Intellectual Property CN925.1
980 Great West Road
BRENTFORD
Middlesex TW8 9GS

Patents ADP number *(if you know it)* 07255006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer yes if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is named as an applicant, or
c) any named applicant is a corporate body
See note (d)

Patents Form 1/77

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Continuation sheets of this form	1
Description	25
Claim(s)	7
Abstract	1
Drawings	6

10. If you are also filing any of the following, state how many against each item.

Priority Documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. We request the grant of a patent on the basis of this application

Signature S C Hockley Date 27-Mar-02

12. Name and daytime telephone number of person to contact in the United Kingdom: S C Hockley 01279 644355

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CONTINUATION SHEET

Reference: SCH/HG/P33025

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Novel Compounds

This invention relates to newly identified diaminoacid-polyamine:peptide and diaminoacid-aminoacid-polyamine based gemini surfactant compounds, to the use of such compounds and to their production. The invention also relates to the use of the diaminoacid-polyamine:peptide based gemini compounds to facilitate the transfer of compounds into cells for drug delivery.

Surfactants are substances that markedly affect the surface properties of a liquid, even at low concentrations. For example surfactants will significantly reduce surface tension when dissolved in water or aqueous solutions and will reduce interfacial tension between two liquids or a liquid and a solid. This property of surfactant molecules has been widely exploited in industry, particularly in the detergent and oil industries. In the 1970s a new class of surfactant molecule was reported, characterised by two hydrophobic chains with polar heads which are linked by a hydrophobic bridge (Deinega,Y *et al.*, *Kolloidn. Zh.* 36, 649, 1974). These molecules, which have been termed "gemini" (Menger, FM and Littau,CA, *J.Am.Chem.Soc.* 113, 1451, 1991), have very desirable properties over their monomeric equivalents. For example they are highly effective in reducing interfacial tension between oil and water based liquids and have a very low critical micelle concentration (Menger, FM and Keiper, JS, *Angewandte. Chem. Int. Ed. Engl.*, 2000, 39, 1906).

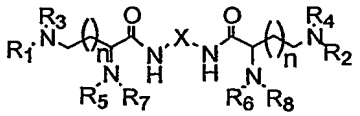
Cationic surfactants have been used *inter alia* for the transfection of polynucleotides into cells in culture, and there are examples of such agents available commercially to scientists involved in genetic technologies (for example the reagent Tfx^{TM}-50 for the transfection of eukaryotic cells available from Promega Corp. WI, USA).

The efficient delivery of DNA to cells *in vivo*, either for gene therapy or for antisense therapy, has been a major goal for some years. Much attention has concentrated on the use of viruses as delivery vehicles, for example adenoviruses for epithelial cells in the respiratory tract with a view to corrective gene therapy for cystic fibrosis (CF). However, despite some evidence of successful gene transfer in CF patients, the adenovirus route remains problematic due to inflammatory side-effects and limited transient expression of the transferred gene. Several alternative methods for *in vivo* gene delivery have been investigated, including studies using cationic surfactants. Gao,X *et al. Gene Ther.* 2, 710-722,1995 demonstrated the feasibility of this approach with a normal human gene for CF transmembrane conductance regulator (CFTR) into the respiratory epithelium of CF mice using amine carrying cationic lipids. This group followed up with a liposomal CF gene therapy trial which, although only partially successful, demonstrated the potential for this approach in humans (Caplen, NJ.

et al., Nature Medicine, 1, 39-46, 1995). More recently other groups have investigated the potential of other cationic lipids for gene delivery (Miller, A, *Angew. Int. Ed. Engl.*, 37, 1768-1785, 1998), for example cholesterol derivatives (Oudrhiri,N et al. *Proc.Natl.Acad.Sci.* 94, 1651-1656, 1997). This limited study demonstrated the ability of these cholesterol based compounds to facilitate the transfer of genes into epithelial cells both *in vitro* and *in vivo*, thereby lending support to the validity of this general approach.

These studies, and others, show that in this new field of research there is a continuing need to develop novel low-toxicity surfactant molecules to facilitate the effective transfer of polynucleotides into cells both *in vitro* for transfection in cell-based experimentation and *in vivo* for gene therapy and antisense treatments. Gemini surfactants based on cysteine (Camilleri, P. *and al.*, patent WO9929712) or on spermine or diamine (Camilleri, P. *and al.*, patent WO0076954) have already been synthesised probing the usefulness of this approach. The present invention seeks to overcome the difficulties exhibited by existing compounds.

The invention relates to diaminoacid-polyamine:peptide based gemini compounds having a diaminoacid-polyamine or a diaminoacid-aminoacid-polyamine backbone and conforming to the general structure of formula (I):

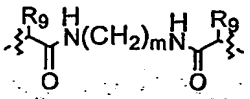
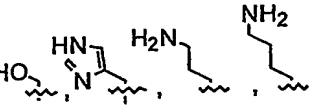


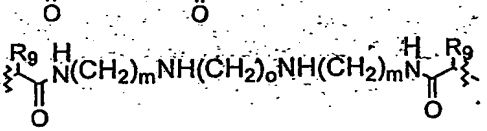
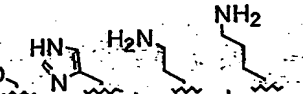
n = 1 to 3

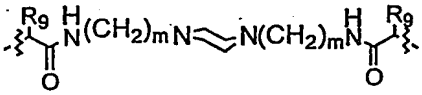
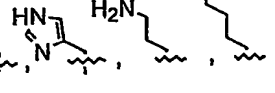
X = -{-$(CH_2)_m$-}- with m = 1 to 10

X = -{-$(CH_2)_mNH(CH_2)_oNH(CH_2)_m$-}- with m = 2 to 5, o = 2 to 5

X = -{-$(CH_2)_mN{=}N(CH_2)_m$-}- with m = 2 to 5

X =  with m = 1 to 10 and R_9 = 

X =  with m = 2 to 5, o = 2 to 5 and R_9 = 

X =  with m = 2 to 5 and R_9 = 

(I)

where diaminoacids relate to aminoacids having two amino groups chosen between α,γ-diaminobutyric acid (n = 1), ornithine (n = 2) and lysine (n = 3) and where polyamines (X) relate to either a linear diaminohydrocarbyl chains having up to 10 carbon atoms (m = 1 to 10)

or

a tetraminohydrocarbyl chain having two terminal amino groups spaced by 2 to 5 carbon atoms (m = 2 to 5) to two internal amino groups spaced in-between by 2 to 5 carbon atoms (o = 2 to 5)

or

a linear aminohydrocarbyl chain having from 2 to 5 carbon atoms length (m = 2 to 5) linked to the nitrogen of a piperazine by an alkyl bond

or

a polyamine as described above linked symmetrically to an amino acid (AA) selected from serine, α-lysine or ε-lysine, ornithine and histidine linked by an amide bond;

and where R_1, R_2, R_3, R_4, R_5 and R_6 is hydrogen and R_7 and R_8 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond;

or

where R_3, R_4, R_5, R_6, R_7 and R_8 is hydrogen and R_1 and R_2 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond;

or

where R_1 and R_2 which may be the same or different are peptide groups formed from one or more amino acids linked together by amide (CONH) bonds and further linked to the diaminoacid-polyamine backbone by amide bonds, in a linear or branched manner, having the general formula (II):

$$-(A1)_{p1}-(A2)_{p2}-(A3)_{p3}$$
$$|$$
$$(A4)_{p4} \qquad \text{(II)}$$

where the values for p1 and p2, which may be the same or different, are from 0 to 5, preferably 1;

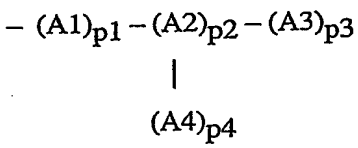
and the values for p3 and p4, which may be the same or different, are from 0 to 5, preferably 0;

A1, A3 and A4, which may be the same or different, is an amino acid selected from serine, lysine, ornithine, threonine, histidine, cysteine, arginine and tyrosine; and

A2 is an amino acid selected from lysine, ornithine and histidine;

and R_3, R_4, R_5 and R_6 are hydrogens and R_7 and R_8 are saturated or unsaturated hydrocarbyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond; or

where R_7 and R_8 which may be the same or different are peptide groups formed from one or more amino acids linked together by amide (CONH) bonds and further linked to the diaminoacid-polyamine backbone by amide bonds, in a linear or branched manner, having the general formula (II):



(II)

where the values for p1 and p2, which may be the same or different, are from 0 to 5, preferably 1; and the values for p3 and p4, which may be the same or different, are from 0 to 5, preferably 0;

A1, A3 and A4, which may be the same or different, is an amino acid selected from serine, lysine, ornithine, threonine, histidine, cysteine, arginine and tyrosine; and

A2 is an amino acid selected from lysine, ornithine and histidine;

and R_3, R_4, R_5 and R_6 are hydrogens and R_1 and R_2 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond; or

a salt, preferably a pharmaceutically acceptable salt thereof.

Preferably, the compound is symmetrical, that is R_1 and R_2 are the same, R_3 and R_4 are the same, R_5 and R_6 are the same, R_7 and R_8 are the same,.

In a preferred embodiment A1 is lysine, serine or threonine, preferably lysine. Preferably A3 and A4 are lysine, ornithine, histidine or arginine.

In a further preferred embodiment the hydrocarboxyl group is selected from:

$-C(O)(CH_2)_{10}CH_3$
$-C(O)(CH_2)_{12}CH_3$
$-C(O)(CH_2)_{14}CH_3$
$-C(O)(CH_2)_{16}CH_3$
$-C(O)(CH_2)_{18}CH_3$
$-C(O)(CH_2)_{20}CH_3$
$-C(O)(CH_2)_7CH=CH(CH_2)_5CH_3$ natural mixture
$-C(O)(CH_2)_7CH=CH(CH_2)_7CH_3$ natural mixture
$-C(O)(CH_2)_7CH=CH(CH_2)_5CH_3$ Cis
$-C(O)(CH_2)_7CH=CH(CH_2)_7CH_3$ Cis
$-C(O)(CH_2)_7CH=CH(CH_2)_5CH_3$ Trans
$-C(O)(CH_2)_7CH=CH(CH_2)_7CH_3$ Trans
$-C(O)(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$
$-C(O)(CH_2)_7(CH=CHCH_2)_3CH_3$
$-C(O)(CH_2)_3CH=CH(CH_2CH=CH)_3(CH_2)_4CH_3$
$-C(O)(CH_2)_7CHCH(CH_2)_7CH_3$
$-C(O)CHCHOH(CH_2)_2CH_3$

In another preferred embodiment the hydrocarboxyl group is selected from:

$-C(O)(CH_2)_{10}CH_3$
$-C(O)(CH_2)_{12}CH_3$
$-C(O)(CH_2)_{14}CH_3$
$-C(O)(CH_2)_{16}CH_3$
$-C(O)(CH_2)_{18}CH_3$
$-C(O)(CH_2)_{20}CH_3$
$-C(O)(CH_2)_{22}CH_3$
$-C(O)(CH_2)_7CH=CH(CH_2)_5CH_3$ natural mixture
$-C(O)(CH_2)_7CH=CH(CH_2)_7CH_3$ natural mixture
$-C(O)(CH_2)_7CH=CH(CH_2)_5CH_3$ Cis
$-C(O)(CH_2)_7CH=CH(CH_2)_7CH_3$ Cis
$-C(O)(CH_2)_7CH=CH(CH_2)_5CH_3$ Trans
$-C(O)(CH_2)_7CH=CH(CH_2)_7CH_3$ Trans
$-C(O)(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$
$-C(O)(CH_2)_7(CH=CHCH_2)_3CH_3$
$-C(O)(CH_2)_3CH=CH(CH_2CH=CH)_3(CH_2)_4CH_3$

Compounds of the present invention may be prepared from readily available starting materials using synthetic peptide chemistry well known to the skilled person. The scheme shown in Figure 1 shows a general scheme for the synthesis of the compounds of the invention wherein the hydrocarboxyl groups are linked to the α-amino group of a diaminoacid further linked to a polyamine backbone moiety by amide bonds, the scheme shown in Figure 2 shows a general scheme for the synthesis of the compounds of the invention wherein the hydrocarboxyl groups are linked to the terminal amino group of a diaminoacid further linked to a polyamine backbone moiety by amide bonds and the scheme shown in Figure 3 shows a general scheme for the synthesis of diaminoacid-aminoacid-polyamine:peptide based gemini compounds wherein an aminoacid is linked by an amide bond to the

amino group (α or terminal) of a diaminoacid further linked to a polyamine moiety by an amide bond.

Another aspect of the invention relates to methods for using the diaminoacid-polyamine:peptide based gemini compounds. Such uses include facilitating the transfer of oligonucleotides and polynucleotides into cells for antisense, gene therapy and genetic immunisation (for the generation of antibodies) in whole organisms. Other uses include employing the compounds of the invention to facilitate the transfection of polynucleotides into cells in culture when such transfer is required, in, for example, gene expression studies and antisense control experiments among others. The polynucleotides can be mixed with the compounds, added to the cells and incubated to allow polynucleotide uptake. After further incubation the cells can be assayed for the phenotypic trait afforded by the transfected DNA, or the levels of mRNA expressed from said DNA can be determined by Northern blotting or by using PCR-based quantitation methods for example the Taqman® method (Perkin Elmer, Connecticut, USA). Compounds of the invention offer a significant improvement, typically between 3 and 6 fold, in the efficiency of cellular uptake of DNA in cells in culture, compared with compounds in the previous art. In the transfection protocol, the gemini compound may be used in combination with one or more supplements to increase the efficiency of transfection. Such supplements may be selected from, for example:

(i) a neutral carrier, for example dioleyl phosphatidylethanolamine (DOPE) (Farhood, H., *et al* (1985) *Biochim. Biophys. Acta,* 1235-1289);

(ii) a complexing reagent, for example the commercially available PLUS reagent (Life Technologies Inc. Maryland, USA) or peptides, such as polylysine or polyornithine peptides or peptides comprising primarily, but not exclusively, basic amino acids such as lysine, ornithine and/or arginine. The list above is not intended to be exhaustive and other supplements that increase the efficiency of transfection are taken to fall within the scope of the invention.

In still another aspect, the invention relates to the transfer of genetic material in gene therapy using the compounds of the invention.

Yet another aspect of the invention relates to methods to effect the delivery of non-nucleotide based drug compounds into cells *in vitro* and *in vivo* using the compounds of the invention.

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Amino acid" refers to dipolar ions (zwitterions) of the form $^{+}H_3NCH®CO_2^{-}$. They are differentiated by the nature of the group R, and when R is different from hydrogen can also be asymmetric, forming D and L families. There are 20 naturally occurring amino acids where the R group can be, for example, non-polar (e.g. alanine, leucine, phenylalanine) or polar (e.g. glutamic acid, histidine, arginine and lysine). In the case of un-natural amino acids R can be any other group which is not found in the amino acids found in nature.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNA's or RNA's containing one or more modified bases and DNA's or RNA's with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications have been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

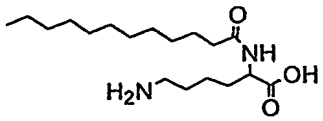
"Transfection" refers to the introduction of polynucleotides into cells in culture using methods involving the modification of the cell membrane either by chemical or physical means. Such methods are described in, for example, Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). The polynucleotides may be linear or circular, single-stranded or double-stranded and may include elements controlling replication of the polynucleotide or expression of homologous or heterologous genes which may comprise part of the polynucleotide.

The invention will now be described by way of the following examples.

EXAMPLES

Example 1:

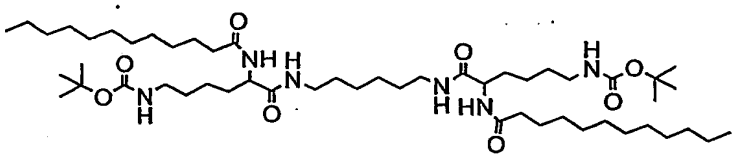
CR-110



To a solution of H-Lys(Boc)-OH (5.02 g, 20.4 mmol) and 20.5 mL of NaOH 1M in 85 Ml of water-acetone (1:2 v/v) cooled at 0°C was added dropwise 4.43 g (20.3 mmol) of dodecyl chloride and NaOH aq. 1M alternatively to maintain the pH over 9. After addition keep 10 minutes more stirring at 0°C. HCl 10% was added until pH 2. Filter the solid and wash with water until pH 7. Dry over P_2O_5. The solid is chromatographied on silica with $CHCl_3$- MeOH to yield 46% of compound CR-110 as a white solid. α_D^{20} -1.0 (c 1.48 , MeOH) ; IR(KBr)ν_{max} 3347, 2921, 2851, 1717, 1681, 1521 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 4.26 (dd, 1H, J= 4.77, 8.92 Hz, C<u>H</u>-COOH), 2.94 (t, 2H, J= 6.7 Hz, CH_2N), 2.16 (t, 2H, J= 7.4 Hz, CH_2CON), 1.78-1.74 (m, 1H, HC<u>H</u>-CH(COOH), 1.63-1.45 (m, 7H, HC<u>H</u>-CH(COOH), C<u>H</u>$_2$C<u>H</u>$_2$N and C<u>H</u>$_2$CH$_2$CON), 1.35 (s, 9H, (C<u>H</u>$_3$)$_3$C), 1.22 (s, 16H, $CH_3(CH_2)_8$), 0.82 (t, 3H, J=6.8 Hz, CH_3); ^{13}C (75 MHz, CD_3OD) 176.32 <u>C</u>(O)NCH$_2$), 175.45 (COOH), 158.42 (C(O)NO), 79.73 (<u>C</u>(CH$_3$)$_3$), 54.84 (CH), 41.16 (CH_2N), 36.97, 33.04, 32.64, 30.74-29.99 (CH_2), 28.83 (CH_3), 26.98, 24.26, 23.71 (CH_2), 14.48 (CH_3).

Example 2:

CR-116

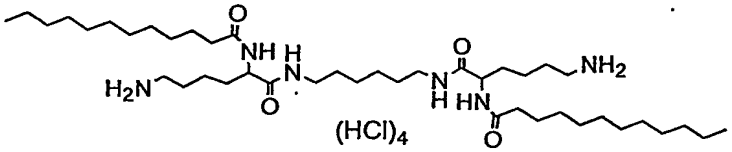


To a solution of 2.4 g (5.6 mmol) of CR-110 in THF at –20°C were added Et_3N (0.78 mL, 5.6 mmol) and EtOCOCl (0,55 mL, 5.6 mmol). The reaction was stirring at this temperature for 30 minutes and 246 mg(2.8 mmol) of 1,4-diaminobutane were added, after 1 hour more stirring at –20°C the reaction mixture was allowed to warm at room temperature and stirred overnight. Remove the solvent in vacuum, the residue was dissolved in $CHCl_3$ and washed with $NaHCO_3$ aq. saturated and brine and dried over MgSO4 anh. The obtained residue was chromatographied to give compound **CR-116** (50%) as a white solid : α_D^{20} -10.06 (c 1.51 , MeOH); IR(KBr)ν_{max} 3415-3307, 2920, 2851, 1688, 1637, 1515 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 4.17 (dd, 1H, J= 5.5, 8.5 Hz, C<u>H</u>-COOH), 3.12 (m,

2H, CH_2N), 2.96 (q, 2H, J= 6.4 Hz, CH_2N), 2.17 (t, 2H, J= 7.4 Hz, $CH_2C(O)N$), 1.69-1.64 (m, 1H, HCH-HC(COOH), 1.58-1.42 (m, 5H, HCH-HC(COOH), CH_2CH_2CO, CH_2CH_2N), 1.36 (s, 9H, $(CH_3)_3C$), 1.22 (s, 16H, $CH_3(CH_2)_8CH_2$), 0.88 (t, 2H, J=6.8 Hz, CH_3); ^{13}C (75 MHz, CD_3OD) 176.26, 174.46 $C(O)NCH_2$), 158.42 ($OC(O)N$), 79.93 ($C(CH_3)_3$), 54.82 (CH), 41.11 and 39.96 (CH_2N), 36.89, 33.09, 32.92, 30.77-30.38 (CH_2), 28.85 (CH_3), 27.63, 26.94, 24.28, 23.74(CH_2), 14.48 (CH_3).

Example 3:

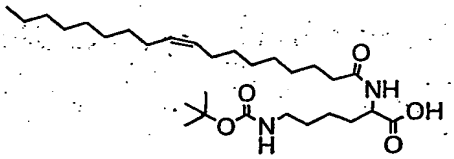
CR-117: GSN11



1.2299 g (1.35 mmol) of CR-116 were treated with EtOAc 4 M for 45 minutes. The solid was filtered and recrystalized from MeOH and EtOAc added to obtain the compound CR-117 as a white solid (49%): α_D^{20} -13.98 (c 1.76 , MeOH); IR(KBr)ν_{max} 3422, 3298, 3089, 2920, 2851, 1638 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 4.20 (dd, 1H, J= 5.6, 8.4 Hz, CH-COOH), 3.12 (m, 2H, CH_2N), 2.84 (t, 2H, J= 6.4 Hz, CH_2N), 2.18 (t, 2H, J= 7.6 Hz, $CH_2C(O)N$), 1.74-1.72 (m, 1H, HCH-CH(COOH), 1.69-1.34 (m, 5H, HCH-CH(COOH) + CH_2CH_2CO+ CH_2CH_2N), 1.22 (s, 16H, $CH_3(CH_2)_8CH_2$), 0.82 (t, 2H, J=6.8 Hz, CH_3); ^{13}C (75 MHz, CD_3OD) 176.39, 174.22 $C(O)NCH_2$), 54.59 (CH), 40.55, 39.99 (CH_2N), 33.08, 32.57, 30.76-30.41(CH_2), 28.23, 27.61, 26.93 (CH_2), 14.44 (CH_3); $C_{40}H_{80}Cl_2N_6O_4 \cdot H_2O$ 778.56 calc C 60.94 %,H 10.36 %, N 10.65 % found C 60.88%, H10.22%, N 10.08%

Example 4

RG 00/781

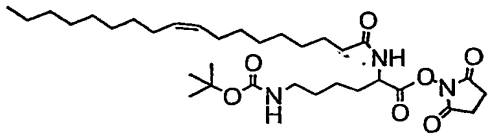


To a solution of *N*-ε-(*tert*butoxycarbonyl)-L-lysine (1.24 g, 5.03 mmol) in THF (140 mL) were added successively a solution of K_2CO_3 (0.75 g, 5.43 mmol, 1.08 eq.) in water (20 mL) and oleoyl succinimidate (1.92 g, 5.06 mmol, 1 eq.). The reaction was stirred at RT for 20 h and most of THF

was evaporated. Water and $CHCl_3$ (30 mL each) were added and the organic layer was separated. The aqueous layer was acidified to pH 2 and extracted twice with $CHCl_3$ (2 x 30 mL). The organic layer was washed with water and brine (20 mL each), dried (Na_2SO_4), filtered and evaporated to give an oil. Yield : 2.46 g (4.82 mmol, **96 %**). ^1H NMR (400 MHz, d_6-DMSO): δ 12.4 (m, 1 H^{OH}), 7.92 (d, 1 H, J = 7.8, HN^{α}), 6.70 (t, 1 H, J = 6.0, HN^{ε}), 5.29 (m, 2 $CH^{9,10}$), 4.10 (dt, 1 H, J = 5.0, 8.9, CH^{α}), 2.85 (q, 2 H, J = 6.2, CH_2^{ε}), 2.07 (dt, 2 H, J = 2.2, 7.0, CH_2^{2}), 1.95 (q, 4 H, J = 6.0, $CH_2^{8,11}$), 1.62 (m, 1 H, CH^{β}), 1.51 (m, 1 H, CH^{β}), 1.45 (m, 2 H, CH_2^{3}), 1.33 (s, 9 H, $C(CH_3)_3$), 1.2 (m, 26 H, 2 $CH_2^{\gamma,\delta}$ and 10 CH_2 oleoyl), 0.82 (t, J = 6.4, 3 H, CH_3^{18}).

Example 5

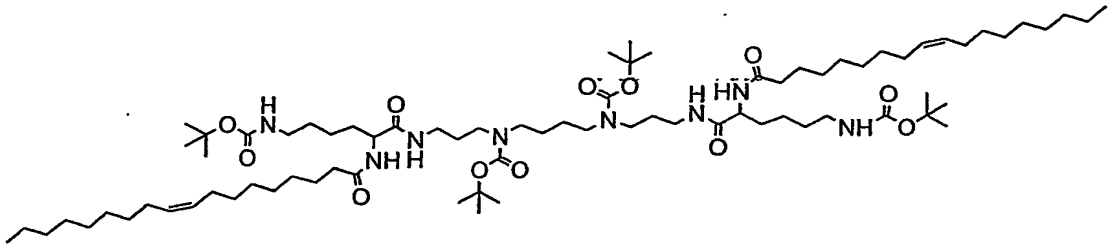
RG 00/366



To a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-Lysine (1.80 g, 3.52 mmol) in THF (80 mL) were added successively *N*-hydroxysuccinimide (0.41 g, 3.56 mmol, 1.01 eq.) and DCC (0.73 g, 3.54 mmol, 1.01 eq.). The reaction was stirred for 16 h at RT. The precipitate was filtered and washed with EtOAc (30 mL). The filtrate was concentrated and redissolved in EtOAc and filtered again. The residue was dissolved in $CHCl_3$ and precipitated with Et_2O to give N-α-oleate-N-ε-(*tert*-butyloxycarbonyl)-L-Lysinyl succinimidate as a white solid. Yield : 1.98 g (**93 %**). NMR ^1H (400 MHz, $CDCl_3$) : δ 6.11 (m, 1 H, HN^{α}), 5.38 (m, 2 H, $H^{9,10}$), 4.94 (m, 1 H, CH^{α}), 4.65 (m, 1 H, HN^{ε}), 3.12 (m, 2 H, CH_2^{ε}), 2.79 (s, 4 H, 2 CH_2^{Su}), 2.20 (t, J = 6.1, 2 H, CH_2^{2}), 2.00 (m, 5 H, CH^{β} and 2 $CH_2^{8,11}$), 1.84 (m, 1 H, CH^{β}), 1.63 (m, 2 H, CH_2^{3}), 1.48 (m, 4 H, 2 $CH_2^{\gamma,\delta}$), 1.37 (s, 9 H, 3 CH_3), 1.27 (m, 20 H, 10 CH_2 oleoyl), 0.83 (t, J = 6.3 Hz, 3 H, CH_3^{18}).

Example 6

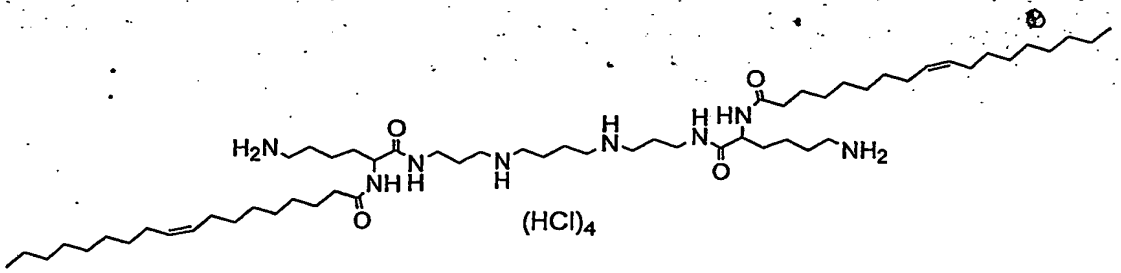
RG 00/250



To a solution of N^4,N^9-bis-(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (629 mg, 1.0 mmol) in THF (80 mL) and K_2CO_3 (0.29 g, 2.1 mmol, 2.1 eq.) in water (10 mL) was added a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysinyl succinimidate (1246 mg, 2.05 mmol, 2.05 eq.). The reaction was stirred overnight at RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 50 mL),. The organic layer was washed with water, 0.1 M HCl, water and brine (20 mL each), dried (Na_2SO_4), filtered, evaporated and purified by column chromatography on SiO_2 ($CHCl_3$ / MeOH : 95/5, Rf = 0.30) to give an oil. Yield : 1060 mg (0.76 mmol, **76 %**). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.30 (bs, 2 H, 2 $NHC^{1'}$), 6.33 (bs, 2 H, 2 NH^{α}), 5.31 (m, 4 H, 2 $CH^{9,10}$), 4.71 (bs, 2 H, 2 NH^{ε}), 4.41 (m, 2 H, 2 CH^{α}), 3.18 (m, 12 H, 2 $CH_2^{1'}$, 2 $CH_2^{3'}$), 3.08 (m, 4 H, 2 $CH_2^{3'}$ and 2 CH_2^{ε}), 2.18 (t, 4 H, J = 6.8, 2 CH_2^{2}), 1.98 (m, 8 H, 2 $CH_2^{8,11}$), 1.90 (m, 2 H, 2 CH^{β}), 1.79 (m, 2 H, 2 CH^{β}), 1.60 (m, 10 H, 2 $CH_2^{2'}$, 2 CH_2^{γ} and 2 CH_2^{3}), 1.45 (m, 26 H, 2 CH_2^{δ}, 2 $CH_2^{5'}$ and 2 $C(CH_3)_3$), 1.40 (s, 18 H, 2 $C(CH_3)_3$), 1.25 (m, 40 H, 2 x 10 $CH_2^{7\text{til}}$), 0.86 (m, 6 H, J = 6.6, 2 CH_3^{18}). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 171.1, 174.3, 155.6, 155.7, 129.5, 129.3, 79.4, 78.5, 76.8, 52.4, 48.6, 46.4, 39.6, 36.1, 33.5, 31.4, 29.3, 29.2, 29.0, 28.8, 28.7, 28.0, 26.7, 25.3, 24.5, 22.2, 22.1, 13.7.

Example 7

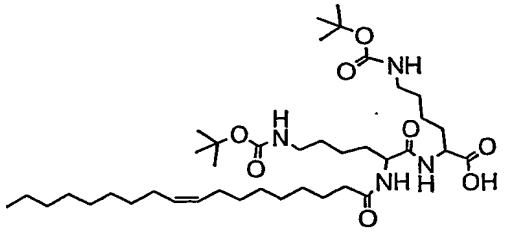
RG 00/267: GSC 102



To a solution of RG 00/250 (1.04 g, 0.75 mmol) in MOH (20 mL) was added concentrated HCl (10 mL) and the reaction was stirred at RT for 2 h. The solvent were then removed and the residue redissolved in water (80 mL), filtered on a frit and evaporated again. The residue was redissolved in a minimum volume of methanol and precipitated with Et_2O to give, after filtration, a pale yellow solid. Yield : 0.734 g (0.65 mmol, **86 %**). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 9.02 (m, 4 H, 2 NH), 8.16 (t, 2 H, J = 6.0, 2 NHC$^{1'}$), 7.98 (s, 6 H, 2 N$^{\alpha}$H and 2 N$^{\varepsilon}$H$_2$), 5.29 (m, 4 H, 2 CH^{9,10}), 4.10 (q, 2 H, J = 7, 2 CH$^{\alpha}$), 3.10 (hp, 4 H, J = 6.4, 2 CH$_2^{1'}$), 2.85 (m, 8 H, 2 CH$_2^{3'}$ and 2 CH$_2^{4'}$), 2.71 (m, 4 H, 2 CH$_2^{\varepsilon}$), 2.10 (AB, 4 H, J = 6.4, 2 CH$_2^{2'}$), 1.95 (m, 8 H, 2 CH$_2^{8,11}$), 1.76 (m, 4 H, 2 CH$_2^{2'}$), 1.68 (m, 4 H, 2 CH$_2^{5'}$), 1.65 – 1.42 (m, 12 H, 2 CH$_2^{\beta}$, 2 CH$_2^{\delta}$ and 2 CH$_2^{3'}$), 1.25 (m, 44 H, 10 CH$_2^{Ol}$ and 2 CH$_2^{\gamma}$), 0.83 (t, 6 H, 2 CH$_3^{18}$). MS (+ES) : 999.8 [M+Na].

Example 8

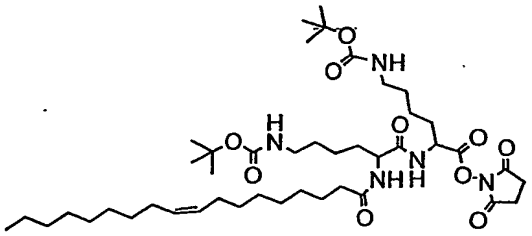
RG00/371



To a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysine (900 mg, 1.48 mmol) in THF (60 mL) were added successively a solution of potassium carbonate (225 mg, 1.63 mmol, 1.1 eq.) in water (6 mL) and *N*-ε-(tert-butyloxycarbonyl)-L-lysine (365 mg, 1.49 mmol, 1 eq.). The solution was then stirred for 16 h at RT. Most of THF was evaporated and pH of the aqueous solution was adjust to 2 and extract with $CHCl_3$ (2 x 80 mL). The organic layer was washed with water (50 mL) and brine (40 mL), dried (Na_2SO_4), filtered and evaporated. The oil obtained was then dissolved in a small quantity of $CHCl_3$ and Et_2O was added. The white solid was then collected. Yield : 1008 mg (1.46 mmol, **99 %**). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 12.60 (m, 1 H, COOH), 8.55 (m, 1 H, NH), 7.10 (m, 1 H, 1 NH), 6.70 (m, 1 H, 1 NH), 5.32 (m, 2 H, CH^{9,10}), 4.80 (m, 1 H, NH), 4.51 (m, 2 H, 2 CH$^{\alpha}$), 3.08 (m, 4 H, 2 CH$_2^{\varepsilon}$), 2.20 (t, 2 H, J = 7.0, 2 CH$_2^{2'}$), 1.99 (m, 4 H, CH$_2^{8,11}$), 1.60 (m, 4 H, 2 CH$_2^{\beta}$), 1.50 – 1.20 (m, 44 H), 0.87 (t, 3 H, J = 6.8, CH$_3^{18}$).

Example 9

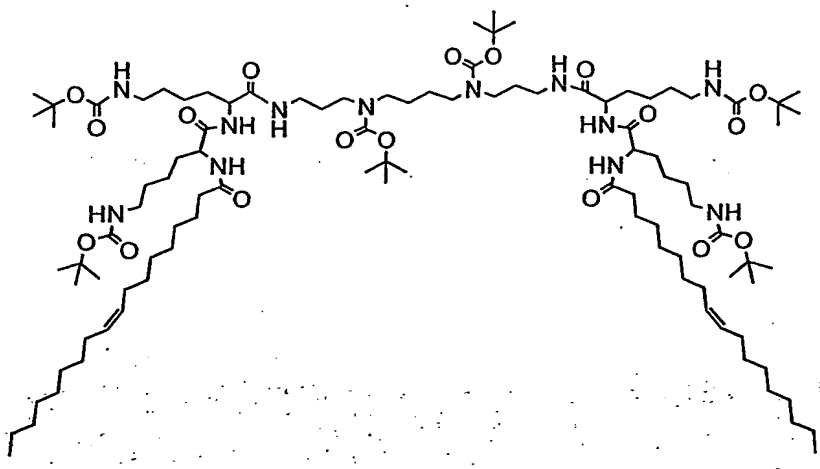
RG 00/376



To a solution of *N*–α-(*N*-α-Oleoyl-*N*-ε-(tert-butyloxycarbonyl)-L-lysyl)-*N*-ε-(tert-butyloxycarbonyl))-L-lysine (1008 mg, 1.46 mmol) in THF (40 mL) was added N-hydroxysuccinimide (177 mg, 1.49 mmol, 1.02 eq.) and DCC (311 mg, 1.50 mmol, 1.03 eq.). The reaction was stirred overnight at RT and the DCU was then filtered and washed with EtOAc. The solvent was then removed and the residue redissolved in EtOAc, the DCU filtered again and after evaporation a white solid was isolated. Yield : 1147 mg (1.36 mmol, 93 %).

Example 10

RG 00/384

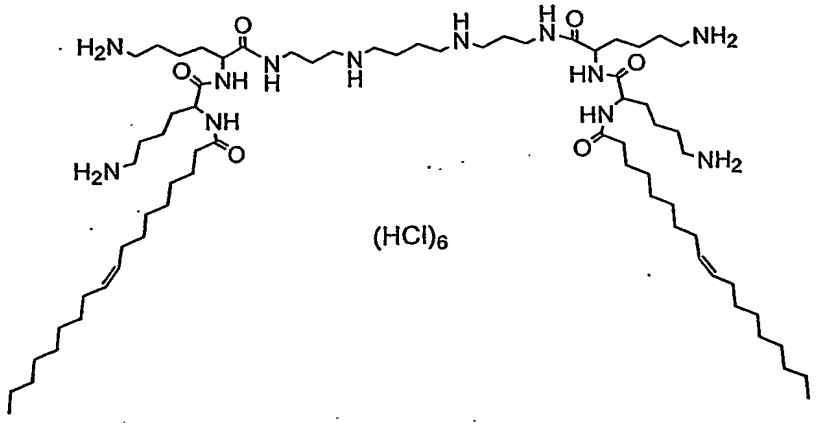


To a solution of N^4,N^9-bis-(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (241 mg, 0.36 mmol) in THF (60 mL) and K_2CO_3 (0.10 g, 0.73 mmol, 2.1 eq.) in water (8 mL) was added a solution of *N*–α-(*N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-Lysyl)-*N*-ε-(tert-butyloxycarbonyl))-L-lysyl succinimidate (600 mg, 0.72 mmol, 2.0 eq.) in THF (10 mL). The reaction was stirred overnight at

RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 60 mL),. The organic layer was washed with water, 0.1 M HCl, water and brine (20 mL each), dried (Na_2SO_4), filtered, evaporated and purified on SiO_2 ($CHCl_3$ / MeOH : 9/1, Rf = 0.27) to give a white solid. Yield : 497 mg (0.27 mmol, 75 %). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.40 (m, 2 H, 2 NH), 6.90 (m, 2 H, 2 NH), 6.40 (m, 2 H, 2 NH), 5.33 (m, 4 H, 2 $CH^{9,10}$), 4.85 (m, 4 H, 4 $N^{\varepsilon}H$), 4.40 (m, 4 H, 2 x 2 CH^{α}), 3.28 – 3.02 (m, 20 H, 2 x 2 CH_2^{ε}, 2 $CH_2^{1'}$, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.22 (m, 4 H, 2 CH_2^{2}), 1.99 (m, 8 H, 2 $CH_2^{8,11}$), 1.80 (m, 4 H, 2 CH_2^{β}), 1.72 – 1.25 (m, 126 H), 0.83 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 11

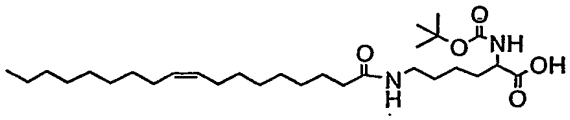
RG 00/404



To a solution of RG 00/384 (470 mg, 0.255 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL) and the reaction was stirred at RT for 1 h. The solvents were removed under vacuum and the residue redissolved into water (80 mL), filtered and evaporated again. The residual oil was dissolved in MeOH and precipitated with Et_2O to give a yellow powder. Yield : 284 mg (0.194 mmol, 76 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 9.10 (m, 4 H, 2 NH_2^+), 8.18 (m, 4 H, *4* NHC), 8.10 – 7.98 (m, 16 H, 2 x 2 $N^{\alpha}H$ and 2 x 2 $N^{\varepsilon}H_3^+$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.18 (m, 2 H, CH^{α}), 4.11 (m, 2 H, 2 CH^{α}), 3.10 (m, 4 H, 2 $CH_2^{1'}$), 2.85 (m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.71 (m, 8 H, 2 x 2 CH_2^{ε}), 2.10 (m, 4 H, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,11}$), 1.80 – 1.39 (m, 28 H), 1.25 (m, 48 H, 10 CH_2^{σ} and 2 x 2 CH_2^{γ}), 0.83 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 12

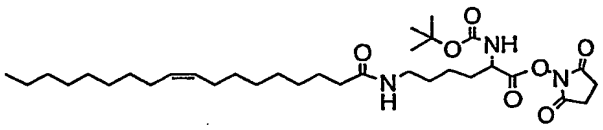
RG 00/278



To a solution of *N*-α-(tert-butyloxycarbonyl)-L-Lysine (779 mg, 3.16 mmol) in THF (80 mL) were added successively a solution of potassium carbonate (0.524 g, 3.79 mmol, 1.2 eq.) in water (10 mL) and oleoyl succinimidate (1.20 g, 3.16 mmol, 1 eq.). The reaction is stirred overnight at room temperature. Most of THF was evaporated and water (40 mL) was added. The aqueous layer was acidified to pH 2 and extracted with $CHCl_3$ (3 x 60 mL). The combined organic layers were washed with water (30 mL) and brine (40 mL), dried over sodium sulphate, filtered and evaporated to give N-α-(tert-butyloxycarbonyl)-N-ε-oleoyl-L-lysine as a colourless oil. Yield : 1.31 g (2.56 mmol, **81 %**). ^{1}H NMR (400 MHz, $CDCl_3$) : d 5.78 (t, 1 H, J = 8.0, NH^{ε}), 5.33 (m, 2 H, $CH^{9,10}$), 5.27 (d, 1 H, J = 7.8, NH^{α}), 4.27 (m, 1 H, CH^{α}), 3.24 (q, 2 H, J = 8.0, CH_2^{ε}), 2.26 (t, 2 H, J = 6.8, CH_2^{2}), 1.98 (m, 4 H, $CH_2^{8,11}$), 1.85 (m, 1 H, CH^{β}), 1.70 (m, 1 H, CH^{β}), 1.60 (m, 2 H, CH_2^{3}), 1.55 (m, 2 H, CH_2^{δ}), 1.43 (s, 9 H, $C(CH_3)_3$), 1.40 (m, 2 H, CH_2^{γ}), 1.27 (m, 20 H, 10 CH_2^{Tail}), 0.87 (m, 3 H, J = 6.6, CH_3^{18}). HRMS (+ES) : 533.40327 calculated for $C_{29}H_{54}O_5N_2Na$ found 533.39110.

Example 13

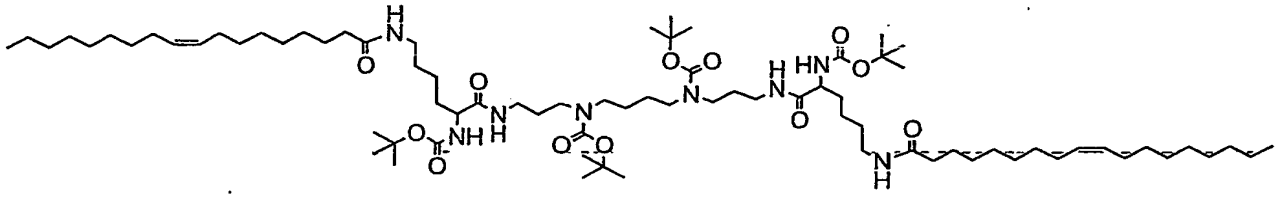
RG 00/281



To a solution of *N*-α-(tert-butyloxycarbonyl)-*N*-ε-oleoyl-L-Lysine (1.80 g, 3.52 mmol) in THF (80 mL) were added successively N-hydroxysuccinimide (0.41 g, 3.56 mmol, 1.01 eq.) and DCC (0.73 g, 3.54 mmol, 1.01 eq.). The reaction was stirred for 16 h at RT. The precipitate was filtered and washed with EtOAc (30 mL). The filtrate was concentrated and redissolved in EtOAc and filtered again. The residue was dissolved in $CHCl_3$ and precipitated with Et_2O to give *N*-α-oleate-*N*-ε-(tert-butyloxycarbonyl)-L-Lysinyl succinimidate as a white solid. Yield : 1.98 g (93 %). ^1H NMR (400 MHz, $CDCl_3$) : d 5.80 (t, 1 H, J = 8.0, NH^{ε}), 5.32 (m, 2 H, $CH^{9,10}$), 5.12 (d, 1 H, J = 7.8, NH^{α}), 4.66 (m, 1 H, CH^{α}), 3.24 (q, 2 H, J = 8.0, CH_2^{ε}), 2.82 (s, 4 H, 2 CH_2^{Su}), 2.14 (t, 2 H, J = 6.8, CH_2^{2}), 1.98 (m, 4 H, $CH_2^{8,11}$), 1.90 (m, 2 H, 2 CH^{β}), 1.60 (m, 2 H, CH_2^{3}), 1.55 (m, 2 H, CH_2^{δ}), 1.44 (s, 9 H, $C(CH_3)_3$), 1.39 (m, 2 H, CH_2^{γ}), 1.25 (m, 20 H, 10 CH_2^{Tail}), 0.86 (m, 3 H, J = 6.6, CH_3^{18}).

Example 14

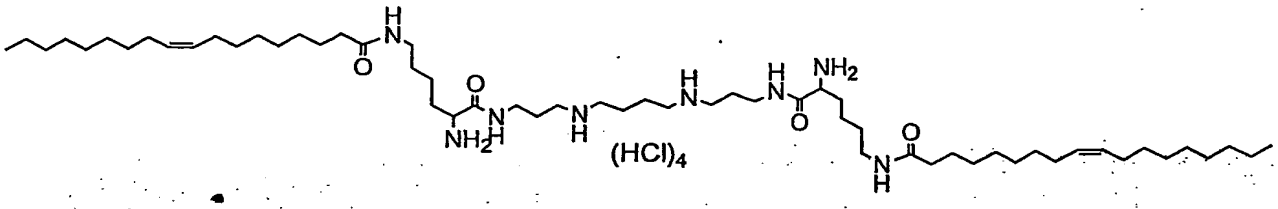
RG 00/286



To a solution of N^{α},N^{9}-bis-(tert-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (414 mg, 0.659 mmol) in THF (60 mL) and K_2CO_3 (200 mg, 1.2 mmol, 2.2 eq.) in water (7 mL) was added a solution of *N*-α-(tert-butyloxycarbonyl)-*N*-ε-oleoyl-L-lysinyl succinimidate (800 mg, 1.32 mmol, 2.0 eq.) in THF (35 mL). The reaction was stirred overnight at RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 30 mL),. The organic layer was washed with water, 0.1 M HCl, water and brine (30 mL each), dried (Na_2SO_4), filtered, evaporated and purified on SiO_2 ($CHCl_3$ / MeOH : 95/5, Rf = 0.27) to give an oil. Yield : 740 mg (0.533 mmol, **81 %**). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.20 (bs, 2 H, 2 NHC$^{1'}$), 5.72 (bs, 2 H, NH$^{\varepsilon}$), 5.33 (m, 4 H, 2 CH^{9,10}), 5.25 (bs, 2 H, 2 NH$^{\alpha}$), 4.08 (m, 2 H, 2 CH$^{\alpha}$), 3.24 (m, 12 H, 2 CH$_2^{1'}$, 2 CH$_2^{4'}$ and 2 CH$_2^{\varepsilon}$), 3.12 (m, 4 H, 2 CH$_2^{3'}$), 2.13 (t, 4 H, J = 6.8, 2 CH$_2^{2}$), 1.98 (m, 8 H, 2 CH$_2^{8,11}$), 1.80 (m, 2 H, 2 CH$^{\beta}$), 1.60 (m, 10 H, 2 CH$_2^{2'}$, 2 CH$^{\beta}$ and 2 CH$_2^{3}$), 1.50 (m, 4 H, 2 CH$_2^{\delta}$), 1.47 (m, 4 H, 2 CH$_2^{5'}$), 1.45 (s, 18 H, 2 C(CH$_3$)$_3$), 1.42 (s, 18 H, 2 C(CH$_3$)$_3$), 1.37 (m, 4 H, 2 CH$_2^{\gamma}$), 1.25 (m, 40 H, 2 x 10 CH$_2^{Tail}$), 0.86 (m, 6 H, J = 6.6, 2 CH$_3^{18}$).

Example 15

RG 00/320 : GSC 101

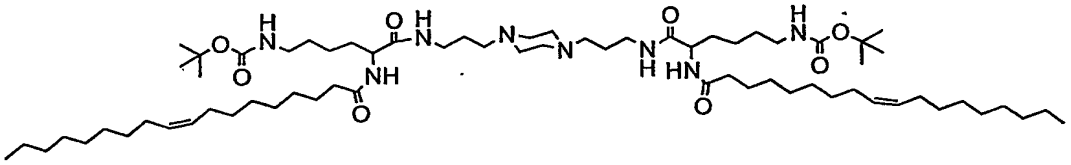


To a solution of RG 00/296 (750 mg, 0.540 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL). The reaction was stirred at RT for 1 h and then evaporated. The residue was redissolved in water (60 mL) and filtered. Water was evaporated and the residue dissolved in a small amount of MeOH and precipitated with Et_2O to give a yellow solid. Yield : 533 mg (0.470 mmol, **90 %**). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 9.02 (m, 4 H, 2 NH_2^+), 8.83 (t, 2 H, *J* = 6.0, 2 NHC$^{1'}$), 8.30 (d, 6 H, *J*

= 4.0, 2 $N^{\alpha}H_3^+$), 8.83 (t, 2 H, J = 6.0, 2 $N^{\varepsilon}H$), 5.30 (m, 4 H, 2 $CH^{9,10}$), 3.70 (q, 2 H, J = 7, 2 CH^{α}), 3.22 (m, 2 H, 2 $CH^{1'}$), 3.13 (m, 2 H, 2 $CH^{1''}$), 2.97 (m, 4 H, 2 CH_2^{8}), 2.71(m, 8 H, 2 $CH_2^{3''}$ and 2 $CH_2^{4''}$), 2.10 (t, 4 H, J = 7.3, 2 CH_2^{2}), 1.95 (q, 8 H, J = 6.0, 2 $CH_2^{8,11}$), 1.82 (h, 4 H, J = 7.0, 2 $CH_2^{2''}$), 1.68 (m, 8 H, 2 CH_2^{β} and 2 $CH_2^{5''}$), 1.43 (qu, 4 H, J = 6.2, 2 CH_2^{3}), 1.35 (m, 4 H, 2 CH_2^{δ}), 1.25 (m, 44 H, 2 x 10 CH_2^{ω} and 2 CH_2^{γ}), 0.82 (t, 6 H, 2 CH_3^{18}). MS (+ES) : 999.8 [M+Na].

Example 16

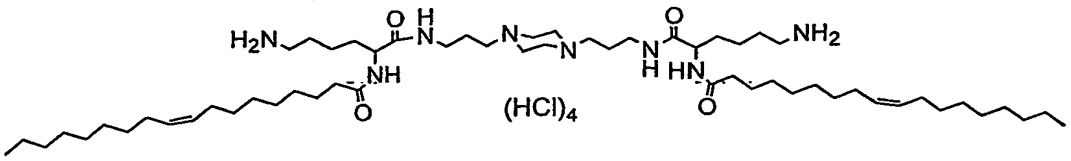
RG 00/518



To a solution of activated aminoacid (610 g, 1.0 mmol) in THF (45 mL) was added bis-*N*-aminopropyl-piperazine (0.081 mL, 0.5 mmol, 0.5 eq.) and then potassium carbonate (0.15 g, 1.1 mmol, 2.2 eq.) in water (10 mL) and the reaction was stirred at RT for 20 h. Most of the THF was removed under vacuum, $CHCl_3$ was added and the organic layer was extracted, washed with water (20 mL), dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography on silica ($CHCl_3$ / MeOH : 8.5 / 1.5, Rf = 0.3) to give a white solid. Yield : 490 mg (0.413 mmol, 83 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (m, 2 H, 2 NHC^{1}), 6.46 (m, 2 H, 2 $N^{\alpha}H$), 5.32 (m, 4 H, 2 $CH^{9,10}$), 4.86 (m, 2 H, 2 N^{ε}Hboc), 4.33 (q, 2 H, J = , 2 CH^{α}), 3.38 (m, 2 H, $CH^{1'}$), 3.28 (m, 2 H, $CH^{1''}$), 3.05 (m, 4 H, 2 CH_2^{8}), 2.47 (m, 12 H, 2 $CH_2^{3''}$ and 4 $CH_2^{2''}$), 2.18 (t, 4 H, J = , 2 CH_2^{2}), 1.99 (m, 8 H, 2 $CH_2^{8,10}$), 1.82 – 1.54 (m, 12 H, 2 $CH_2^{2''}$, 2 CH_2^{3} and 2 CH_2^{β}), 1.48 (m, 4 H, 2 CH_2^{γ}), 1.42 (s, 18 H, 2 $(CH_3)_3$), 1.21 (m, 24 H, 10 CH_2^{ω} and 2 CH_2^{γ}), 0.87 (t, 6 H, J = 6.4, 2 CH_3^{18}). ^{13}C NMR (400 MHz, CD_3OD): δ 175.2, 173.4, 157.5, 129.9, 129.8, 78.8, 56.0, 53.8, 52.9, 41.3, 40.1, 37.7, 35.9, 32.1, 31.9, 29.9, 29.6, 29.5, 29.4, 29.3, 27.9, 27.2, 26.2, 26.0, 23.3, 22.8, 13.5.

Example 17

RG 00/522 = GSC 170

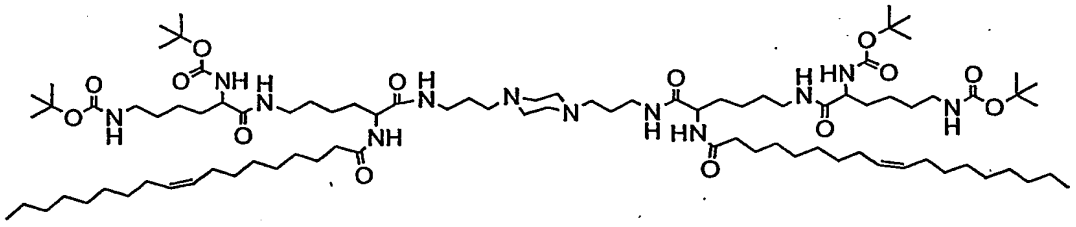


To a solution of protected RG00/518 (490 mg, 0.413 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL). The reaction was stirred for 1 h and the solvent was then evaporated. The residue was redissolved in water (40 mL), filtered and evaporated. In this case it was impossible to precipitate the compound using MeOH / Et_2O. A white solid was collected. Yield : 381 mg (0.337 mmol, **81 %**). HRMS (+ES) : 985.8879 calculated for $C_{58}H_{113}N_8O_4$, found 985.8890.

Note : a similar procedure using TFA and neutralisation with K_2CO_3 was used to isolate the free amine in a quantitative yield. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.78 (2 d, 4 H, J = 8.0, 4 NHCO), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.12 (q, 2 H, J = 6.2, 2 CH^{α}), 3.04 (m, 4 H, 2 $CH_2^{1'}$), 2.47 (m, 8 H, 4 CH_2^{4}), 2.29 (m, 4 H, 2 NH_2), 2.19 (t, 4 H, J = 6.2, 2 $CH_2^{3'}$), 2.05 (m, 4 H, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.35 – 1.69 (m, 12 H, 2 $CH_2^{2'}$, 2CH_2^{3} and 2 CH_2^{β}), 1.21 (m, 26 H, 10 CH_2^{01} and CH_2^{δ} and CH_2^{γ}), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 18

RG 00/794

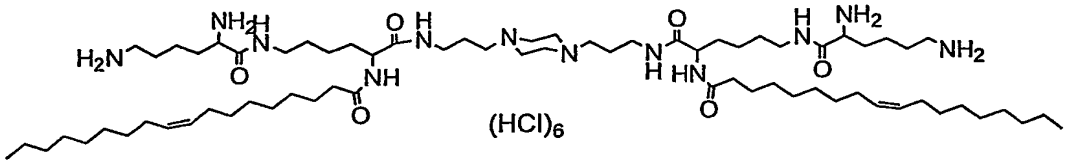


To a solution of bis aminocompound (150 mg, 0.152 mmol) in THF (40 mL) was added successively a solution of K_2CO_3 (42 mg, mmol, 2.1 eq.) in water (2 mL) and *N,N*-bis-(tertbutoxycarbonyl)-L-lysinyl succinimidate (140 mg, 0.304 mmol, 2.0 eq.) in THF (10 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer extracted, washed with water (2 x 10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue is purified on SiO_2 (eluent : $CHCl_3$ / MeOH /

NH_4OH: 87 / 12 / 1, Rf = 0.28). Et_2O is then added and the resulting white solid filtered off. Yield : 0.124 g (0.076 mmol, **50 %**). ^1H NMR (400 MHz, d^6-DMSO) : δ 7.75 (m, 4 H, 2 $NH^{\alpha 1}$ and 2 $NHC^{1'}$), 7.68 (t, 2 H, J = , 2 $NH^{\varepsilon 1}$), 6.69 (t, 2 H, J = , 2 $NH^{\varepsilon 2}$), 6.63 (d, 2 H, J = , 2 $NH^{\alpha 2}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.10 (q, 2 H, J = , 2 $CH^{\alpha 1}$), 3.78 (q, 2 H, J = , 2 $CH^{\alpha 2}$), 3.00 (m, 6 H, 2 $CH_2^{\varepsilon 1}$ and 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.84 (m, 4 H, 2 $CH_2^{\varepsilon 2}$), 2.29 (m, 8 H, 4 $CH_2^{4'}$), 2.19 (m, 4 H, 2 $CH_2^{3'}$), 2.06 (t, 4 H, J = , 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.55 – 1.4 (m, 16 H), 1.32 (s, 36 H, 4 $C(CH_3)_3$), 1.20 (m, 48 H), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 19

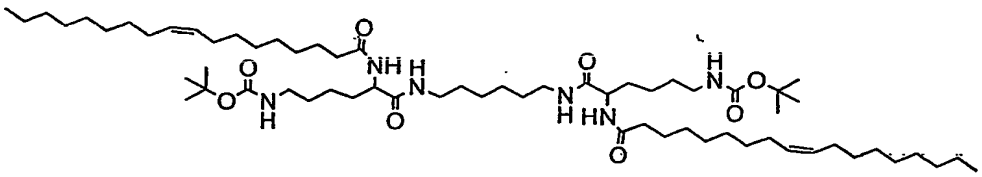
RG00/813 = GSC 184



To a solution of RG00/794 (124 mg, 0.0755 mmol) in MeOH (5 mL) was added concentrated HCl (5 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vacuum. The residue was dissolved in water, filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected. Yield : 0.102 g (0.070 mmol, **93 %**). ^1H NMR (400 MHz, d_6-DMSO) : δ 8.66 (d, 2 H, J = 7.8, 2 $NH^{\varepsilon 1}$), 8.28 (m, 6 H, 2 $N^{\alpha}H_3^+$), 8.09 (m, 2 H, 2 $NHC^{1'}$), 8.05 (m, 6 H, 2 $N^{\varepsilon}H_3^+$), 7.98 (d, 2 H, J = 7.0, 2 $N^{\alpha}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.09 (m, 2 H, 2 $CH^{\alpha 1}$), 3.72 (m, 2 H, 2 $CH^{\alpha 2}$), 3.65 (m, 2 H, 2 NH^+), 3.10 (m, 12 H, 2 $CH_2^{\varepsilon 1}$, 2 $CH_2^{3'}$ and 2 $CH_2^{1'}$), 2.74 (m, 8 H, 2 $CH_2^{\varepsilon 2}$), 2.11 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.82 (m, 2 H, 2 $CH_2^{\delta 1}$), 1.70 (m, 2 H, 2 $CH_2^{\beta 2}$), 1.57 (m, 6 H, 2 $CH_2^{\delta 2}$ and 2 $CH^{\beta 1}$), 1.50 – 1.15 (m, 66 H), 0.84 (t, 6 H, J = 6.4, 2 CH_3^{18}). MS (+ES) : 1264.9 [M+Na].

Example 20

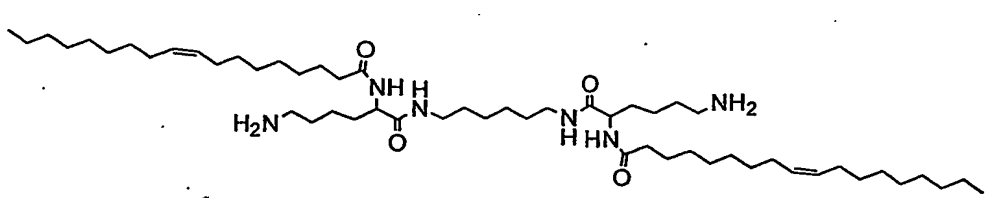
RG 00/787



To a solution of 1,6-diaminohexane (72 mg, 0.62 mmol) in THF (60 mL) and K_2CO_3 (180 mg, 1.30 mmol, 2.1 eq.) in water (10 mL) was added a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysinyl succinimidate (750 mg, 1.23 mmol, 2 eq.). The reaction was stirred overnight at RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 50 mL). The organic layer was washed with water, 0.1 M HCl, water and brine (20 mL each), dried (Na_2SO_4), filtered, evaporated and purified by column chromatography on SiO_2 ($CHCl_3$ / MeOH : 9/1, Rf = 0.33) to give an oil. Yield : 650 mg (0.59 mmol, **95 %**). ^1H NMR (400 MHz, d_6-DMSO) : δ 7.73 (m, 4 H, 2 $N^{\alpha}H$ and 2 $N^{1'}H$), 6.68 (t, 2 H, *J* = 5.0, 2 $N^{\varepsilon}H$), 5.28 (m, 4 H, 2 $CH^{9,10}$), 4.12 (m, 2 H, 2 CH^{α}), 2.99 (q, 4 H, J = 6.4, 2 $CH^{1'}$), 2.83 (q, 4 H, *J* = 6.6, 2 CH_2^{ε}), 2.07 (dt, 4 H, *J* = 3.2, 7.0, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,11}$), 1.52 (m, 2 H, 2 CH^{β}), 1.42 (m, 6 H, 2 CH_2^{3} and 2 CH^{β}), 1.32 (s, 18 H, 2 $C(CH_3)_3$), 1.31 – 1.15 (m, 56 H, 2 x 10 CH_2^{Tail}, 2 CH_2^{δ}, 2 CH_2^{γ}, 2 $CH_2^{2'}$ and 2 $CH_2^{3'}$), 0.82 (t, 6 H, *J* = 6.8, 2 CH_3^{18}).

Example 21

RG 00/873: GSN 14

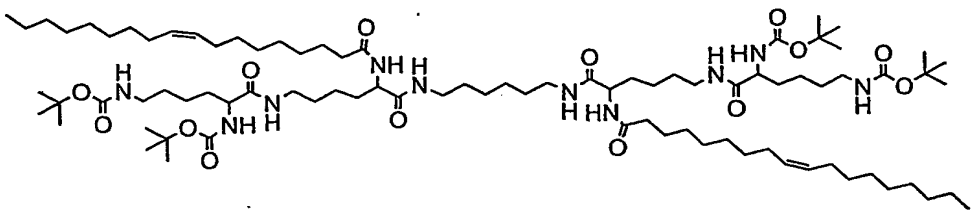


To a solution of protected compound (640 mg, 0.581 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL). The reaction was stirred at RT for 1 h and then evaporated (using several Et_2O (10 mL) to coevaporate). The oily residue was then dissolved in CH_2Cl_2, washed with 10 % aqueous K_2CO_3 (10 mL), water and brine. The organic phase was dried (Na_2SO_4), filtered and evaporated to give a pale brown solid which was triturated with Et_2O, filtered and dried to give a white solid. Yield : 460 mg (0.510 mmol, 88 %). The deprotection can be carried out using concentrated HCl in methanol giving

the hydrochloric salt named GSN 14. ^{1}H NMR (400 MHz, d_6-DMSO) : δ 7.80 (m, 4 H, 2 $N^{\alpha}H$ and 2 $N^{1'}H$), 5.28 (m, 4 H, 2 $CH^{9,10}$), 4.16 (m, 2 H, 2 CH^{α}), 3.20 (bs, 4 H, 2 NH_2), 2.99 (q, 4 H, J = 6.4, 2 $CH^{1'}$), 2.53 (m, 4 H, 2 CH_2^{6}), 2.10 (dt, 4 H, J = 3.2, 7.0, 2 CH_2^{2}), 1.91 (m, 8 H, 2 $CH_2^{8,11}$), 1.52 (m, 2 H, 2 CH^{β}), 1.48 (m, 2 H, 2 CH^{β}), 1.42 (m, 4 H, 2 CH_2^{3}), 1.31 – 1.15 (m, 56 H, 2 x 10 CH_2^{Tail}, 2 CH_2^{δ}, 2 CH_2^{γ}, 2 $CH_2^{2'}$ and 2 $CH_2^{3'}$), 0.81 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 22

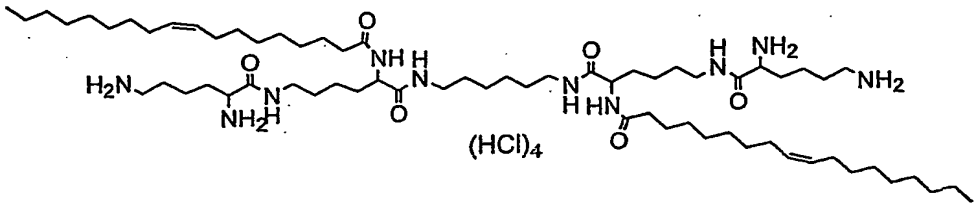
RG 00/874



To a 1/9 mixture of water and THF (20 mL) containing RG 00/873 (100 mg, 0.111 mmol) and potassium carbonate (32 mg, 0.232 mmol, 2.1 eq.) was added *N,N*-bis-(tert-butyloxycarbonyl)-L-lysinyl succinimidate (103 mg, 0.232 mmol, 2.1 eq.). The reaction was stirred for 20 h at RT. Most of THF was removed and the residue diluted with water (10 mL) and $CHCl_3$ (40 mL). The organic layer was decanted and washed successively with water (10 mL), 0.1 M HCl (20 mL), water (10 mL) and brine (25 mL). The organic layer was dried over sodium sulphate, filtered and evaporated. The resulting oil was crystallised from Et_2O. The white solid was collected. Yield : 164 mg (0.105 mmol, **95 %**).

Example 23

RG 00/875 = GSC 197



To a solution of RG 00/874 (160 mg, 0.103 mmol) in methanol (5 mL) is added concentrated HCl (5 mL). The reaction is stirred for 1 h and then evaporated to dryness. The residue is then dissolved in water (30 mL), filtered on sintered frit funnel (N° 3), evaporated to dryness using EtOH to coevaporate. The residue is dissolved in a small amount of methanol and precipitated with Et_2O to give the desired compound as a pale brown solid. Yield : 124 mg (0.951 mmol, **95 %**). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.59 (t, 2 H, J = 5.0, 2 $N^{1'}H$), 8.22 (m, 6 H, 2 $N^{\alpha 2}H_3^+$), 7.96 (m, 6 H, 2 $N^{\varepsilon 2}H_3^+$), 7.89 (d, 2 H, J = 8.0, 2 $N^{\alpha 1}H$), 7.89 (t, 2 H, J = 5.8, 2 $N^{\varepsilon 1}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.14 (dt, 2 H, J = 5.4, 8.0, 2 $CH^{\alpha 1}$), 3.70 (m, 2 H, 2 $CH^{\alpha 2}$), 3.05 (m, 4 H, 2 $CH_2^{1'}$), 2.98 (q, 4 H, J = 5.8, 2 $CH_2^{\varepsilon 1}$), 2.72 (m, 4 H, 2 $CH_2^{\varepsilon 2}$), 2.09 (t, 4 H, J = 7.0, 2 CH_2^{2}), 1.94 (m, 8 H, 2 $CH_2^{8,11}$), 1.69 (m, 2 H, 2 $CH_2^{\beta 2}$), 1.55 (m, 6 H, 2 $CH_2^{\delta 2}$ and 2 $CH^{\beta 1}$), 1.48 – 1.15 (m, 56 H), 0.81 (t, 6 H, J = 6.6, 2 CH_3^{18}).

Example 24. Transfection of recombinant plasmid expressing luciferase into cells using lysine-polyamine-based gemini compounds.

Transfection studies were performed using the adherent cell line CHO-K1 (Puck et al. 1958). Complete medium consisted of MEM alpha medium supplemented with 10 % v/v foetal bovine serum and 1x L-Glutamine. All media and supplements were obtained from Life Technologies.

Stable transfected cell lines expressing β–galactosidase were generated by cotransfection of the plasmid pSV-β-Galactosidase Control Vector (Promega) with the plasmid Selecta Vecta-Neo (R & D Systems) in a 10:1 ratio. Following G418 (Life Technologies) selection (0.8 mg ml^{-1}), candidate cell lines were tested for β–galactosidase activity (β–Gal Reporter Gene Assay, chemiluminescent; Roche Diagnostics).

***In Vitro* Gene Transfection.**

Cells were seeded into 96-well MTP plates (Nunc) 16-18 hours prior to transfection at an approximate density of 1 x 10^4 cells per well. For transfection, 0.064 μg of the luciferase reporter gene plasmid, pGL3-Control Vector (Promega) per well, was incubated with various concentrations

of the diaminoacid-polyamine:peptide-based gemini compounds and complexing agents in a final volume of 100 µl. After 30 minutes incubation at RT, OPTI-MEM® medium (Life Technologies) was added to the transfection mixture and the solution placed on the cells (final volume per well: 100 µl). Following a 3 hour or over night incubation at 37°C, the transfection solution was replaced with complete medium and the cells incubated further at 37°C. Reporter gene assays were performed according to the manufacturer's guidelines (Roche Diagnostics) approximately 48 hours post transfection. Luminescence was measured in a Packard TopCount NXT Microplate Scintillation and Luminescence Counter. For normalisation purpose, β–galactosidase activity (β–Gal Reporter Gene Assay, chemiluminescent; Roche Diagnostics) was measured and luciferase activity per β–galactosidase activity was calculated.

Brief description of the drawings

Figure 1. General scheme for synthesis of diaminoacid-polyamine:peptide based gemini compounds wherein the hydrophobic tail is linked to the α-amino group of a diaminoacid further linked to a polyamine moiety by amide bonds.

Figure 2. General scheme for synthesis of diaminoacid-polyamine:peptide based gemini compounds wherein the hydrophobic tail is linked to the terminal amino group of a diaminoacid further linked to a polyamine moiety by amide bonds.

Figure 3. General scheme for the synthesis of diaminoacid-aminoacid-polyamine:peptide based gemini compounds wherein an aminoacid is linked by an amide bond to the α-amino group of a diaminoacid further linked to a polyamine moiety by amide bonds.

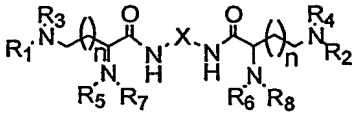
Figure 4. Transfection of CHO-K1 cells (stable transfected with beta-galactosidase) with Gemini surfactants. Bars represent the mean cps (counts per second) of 8 experiments ± the standard error of the mean.

Figure 5. Transfection of CHO-K1 cells (stable transfected with beta-galactosidase) with Gemini surfactants. Bars represent the mean cps (counts per second) of 8 experiments ± the standard error of the mean.

Figure 6. Transfection of CHO-K1 cells (stable transfected with beta-galactosidase) with Gemini surfactants. Bars represent the mean cps (counts per second) of 8 experiments ± the standard error of the mean.

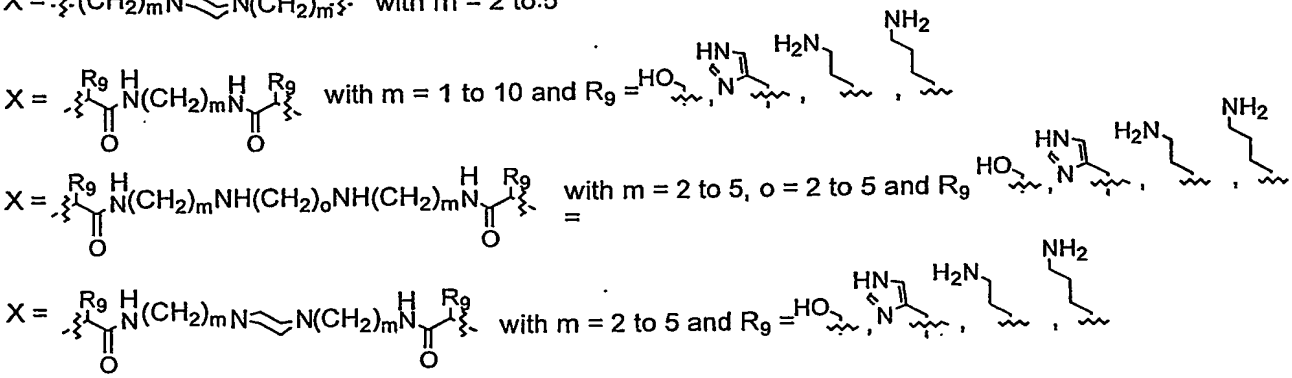
CLAIMS

1. A peptide-diaminoacid-polyamine-based gemini compounds having a diaminoacid-polyamine backbone and conforming to the general structure of formula (I):



n = 1 to 3

X = -(CH$_2$)$_m$- with m = 1 to 10
X = -(CH$_2$)$_m$NH(CH$_2$)$_o$NH(CH$_2$)$_m$- with m = 2 to 5, o = 2 to 5
X = -(CH$_2$)$_m$N=N(CH$_2$)$_m$- with m = 2 to 5



(I)

where diaminoacids relate to aminoacids having two amino groups chosen between α,γ-diaminobutyric acid (n = 1), ornithine (n = 2) and lysine (n = 3) and where polyamine (X) relates to either a linear diaminohydrocarbyl chains having up to 10 carbon atoms (m = 1 to 10)

or

a tetraminohydrocarbyl chain having two terminal amino groups spaced by 2 to 5 carbon atoms (m = 2 to 5) to two internal amino groups spaced in-between by 2 to 5 carbon atoms (o = 2 to 5)

or

a linear aminohydrocarbyl chain having from 2 to 5 carbon atoms length (m = 2 to 5) linked to the nitrogen of a piperazine by an alkyl bond

or

a polyamine as described above linked symmetrically to an amino acid selected from serine, lysine, ornithine and histidine;

and where R_1, R_2, R_3, R_4, R_5 and R_6 is hydrogen and R_7 and R_8 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond;

or

where R_3, R_4, R_5, R_6, R_7 and R_8 is hydrogen and R_1 and R_2 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond;

or

where R_1 and R_2 which may be the same or different are peptide groups formed from one or more amino acids linked together by amide (CONH) bonds and further linked to the diaminoacid-polyamine backbone by amide bonds, in a linear or branched manner, having the general formula (II):

$$\begin{array}{l} -(A1)_{p1}-(A2)_{p2}-(A3)_{p3} \\ \qquad\qquad\quad | \\ \qquad\quad\;\; (A4)_{p4} \end{array} \qquad \text{(II)}$$

where the values for p1 and p2, which may be the same or different, are from 0 to 5, preferably 1; and the values for p3 and p4, which may be the same or different, are from 0 to 5, preferably 0; A1, A3 and A4, which may be the same or different, is an amino acid selected from serine, lysine, ornithine, threonine, histidine, cysteine, arginine and tyrosine; and A2 is an amino acid selected from lysine, ornithine and histidine; and R_3, R_4, R_5 and R_6 are hydrogens and R_7 and R_8 are saturated or unsaturated hydrocarbyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond;

or

where R_7 and R_8 which may be the same or different are peptide groups formed from one or more amino acids linked together by amide (CONH) bonds and further linked to the diaminoacid-polyamine backbone by amide bonds, in a linear or branched manner, having the general formula (II):

$$\begin{array}{l} -(A1)_{p1}-(A2)_{p2}-(A3)_{p3} \\ \qquad\qquad\quad | \\ \qquad\quad\;\; (A4)_{p4} \end{array} \qquad \text{(II)}$$

where the values for p1 and p2, which may be the same or different, are from 0 to 5, preferably 1; and the values for p3 and p4, which may be the same or different, are from 0 to 5, preferably 0;
A1, A3 and A4, which may be the same or different, is an amino acid selected from serine, lysine, ornithine, threonine, histidine, cysteine, arginine and tyrosine; and
A2 is an amino acid selected from lysine, ornithine and histidine;
and R_3, R_4, R_5 and R_6 are hydrogens and R_1 and R_2 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond; or
a salt, preferably a pharmaceutically acceptable salt thereof.

2. A diaminoacid-polyamine:peptide-based gemini compound according to claim 1 which is symmetrical, that is R_1 and R_2 are the same, R_3 and R_4 are the same, R_5 and R_6 are the same and R_7 and R_8 are the same.

3. A diaminoacid-polyamine:peptide-based gemini compound according to claim 1 or 2 wherein in the peptide group of formula (II) p1 and p2 are both 1 and p3 and p4 are both 0.

4. A diaminoacid-polyamine:peptide-based gemini compound according to any one of claims 1 to 3 wherein the A1 is lysine.

5. A diaminoacid-polyamine:peptide-based gemini compound according to any one of claims 1 to 4 wherein the A2 is lysine.

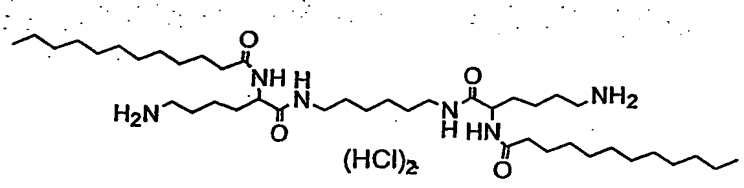
6. A diaminoacid-polyamine:peptide-based gemini compound according to claim 1 wherein the hydrocarboxyl group is selected from:

-C(O)$(CH_2)_{10}CH_3$
-C(O)$(CH_2)_{12}CH_3$
-C(O)$(CH_2)_{14}CH_3$
-C(O)$(CH_2)_{16}CH_3$
-C(O)$(CH_2)_{18}CH_3$
-C(O)$(CH_2)_{20}CH_3$
-C(O)$(CH_2)_7CH{=}CH(CH_2)_5CH_3$ natural mixture
-C(O)$(CH_2)_7CH{=}CH(CH_2)_7CH_3$ natural mixture
-C(O)$(CH_2)_7CH{=}CH(CH_2)_5CH_3$ Cis
-C(O)$(CH_2)_7CH{=}CH(CH_2)_7CH_3$ Cis
-C(O)$(CH_2)_7CH{=}CH(CH_2)_5CH_3$ Trans
-C(O)$(CH_2)_7CH{=}CH(CH_2)_7CH_3$ Trans
-C(O)$(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$
-C(O)$(CH_2)_7(CH{=}CHCH_2)_3CH_3$
-C(O)$(CH_2)_3CH{=}CH(CH_2CH{=}CH)_3(CH_2)_4CH_3$
-C(O)$(CH_2)_7CHCH(CH_2)_7CH_3$
-C(O)$CHCHOH(CH_2)_2CH_3$

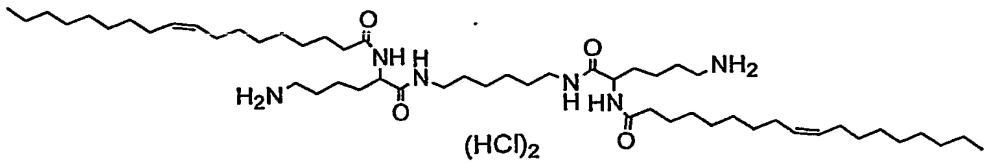
7. A diaminoacid-polyamine based gemini compound according to claim 1 wherein the hydrocarboxyl group is selected from:

-C(O)$(CH_2)_{10}CH_3$
-C(O)$(CH_2)_{12}CH_3$
-C(O)$(CH_2)_{14}CH_3$
-C(O)$(CH_2)_{16}CH_3$
-C(O)$(CH_2)_{18}CH_3$
-C(O)$(CH_2)_{20}CH_3$
-C(O)$(CH_2)_{22}CH_3$
-C(O)$(CH_2)_7CH{=}CH(CH_2)_5CH_3$ natural mixture
-C(O)$(CH_2)_7CH{=}CH(CH_2)_7CH_3$ natural mixture
-C(O)$(CH_2)_7CH{=}CH(CH_2)_5CH_3$ Cis
-C(O)$(CH_2)_7CH{=}CH(CH_2)_7CH_3$ Cis
-C(O)$(CH_2)_7CH{=}CH(CH_2)_5CH_3$ Trans
-C(O)$(CH_2)_7CH{=}CH(CH_2)_7CH_3$ Trans
-C(O)$(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$
-C(O)$(CH_2)_7(CH{=}CHCH_2)_3CH_3$
-C(O)$(CH_2)_3CH{=}CH(CH_2CH{=}CH)_3(CH_2)_4CH_3$

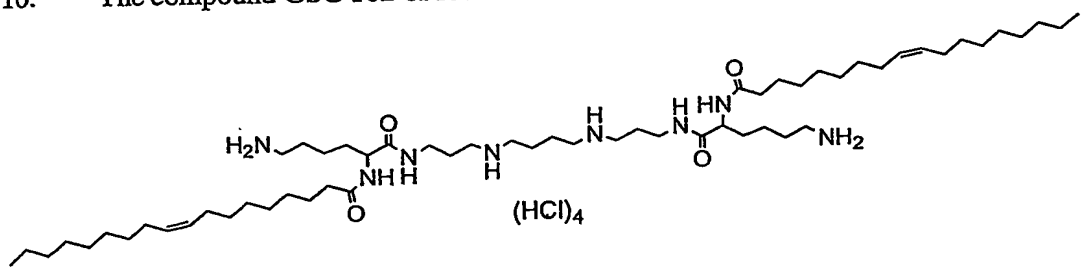
8. The compound GSN 11 of formula:



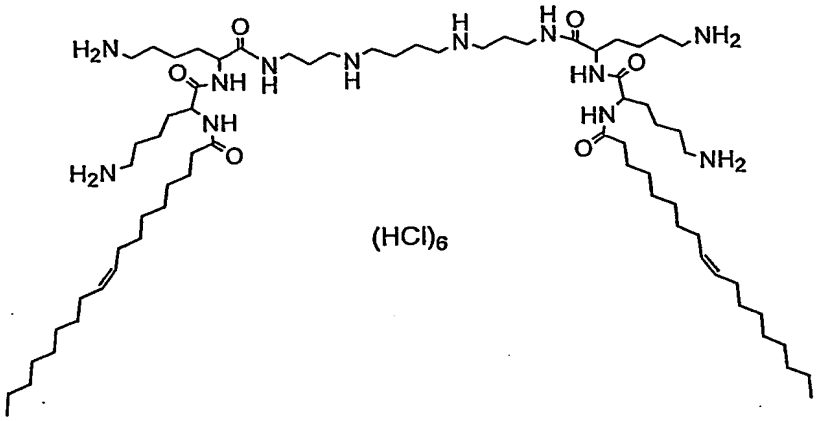
9. The compound GSN 14 of formula:



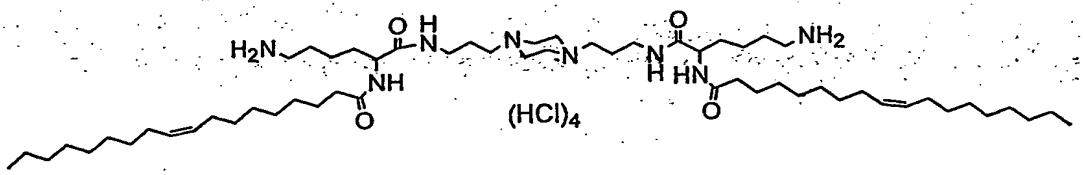
10. The compound GSC 102 of formula:



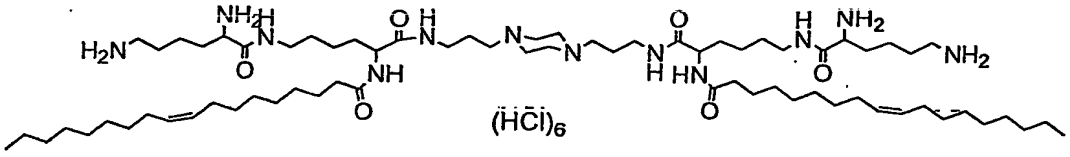
11. The compound GSC 157 of formula:



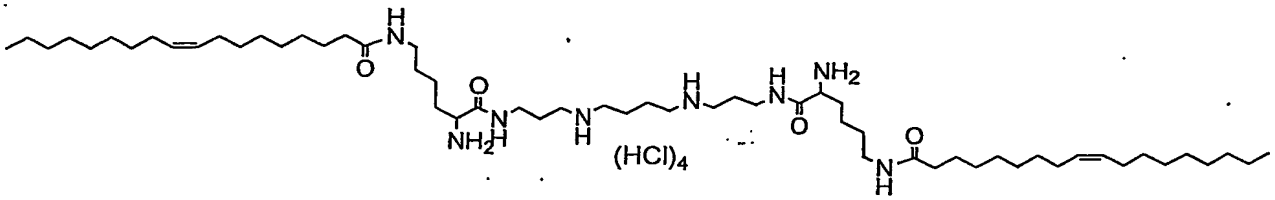
12. The compound GSC170 of formula:



13. The compound GSC 184 of formula:



14. The compound GSC101 of formula:



15. The use of a diaminoacid-polyamine:peptide-based gemini compound as defined in any one of claims 1 to 14 in enabling transfection of DNA or RNA or analogues thereof into a eukaryotic or prokaryotic cell *in vivo* or *in vitro*.

16. The use of a diaminoacid-polyamine:peptide-based gemini compound according to claim 15 wherein the compound is used in combination with one or more supplements selected from the group consisting of:

(i) a neutral carrier; or

(ii) a complexing reagent.

17. The use according to claim 16 wherein the neutral carrier is dioleyl phosphatidylethanolamine (DOPE).

18. The use according to claim 17 wherein the complexing reagent is PLUS reagent.

19. The use according to claim 18 wherein the complexing reagent is a peptide comprising mainly basic amino acids.

20. The use according to claim 19 wherein the peptide consists of basic amino acids.

21. The use according to claim 19 or 20 wherein the basic amino acids are selected from lysine and arginine.

22. The use according to claim 20 wherein the peptide is polylysine or polyornithine.

23. A method of transfecting polynucleotides into cells *in vivo* for gene therapy, which method comprises administering diaminoacid-polyamine:peptide-based gemini compounds of any one of claims 1 to 22 together with, or separately from, the gene therapy vector.

24. The use of a diaminoacid-polyamine-based gemini compound of any one of claims 1 to 14 to facilitate the transfer of a polynucleotide or an anti-infective compounds into prokaryotic or eukaryotic organism for use in anti-infective therapy.

25. The use of a diaminoacid-polyamine-based gemini compound of any one of claims 1 to 14 to facilitate the adhesion of cells in culture to each other or to a solid or semi-solid surface.

32. A process for preparing diaminoacid-polyamine-based gemini compounds of claim 1 or 2 which process comprises the coupling of a succinimidate ester of a diaminoacid linked to its α or terminal amino group to an hydrocarboxyl chain to a polyamine linker using potassium carbonate as a base in a mixture of tetrahydrofuran and water as solvents.

Abstract

Diaminoacid-polyamine:peptide-based gemini compounds are disclosed. The compounds are based on diaminoacid-polyamine or diaminoacid-aminoacid-polyamine backbone with peptide groups and optionally hydrocarboxyl groups linked thereto. Uses of the Diaminoacid-polyamine:peptide-based gemini compounds and methods for their production are also disclosed.

Figure 1

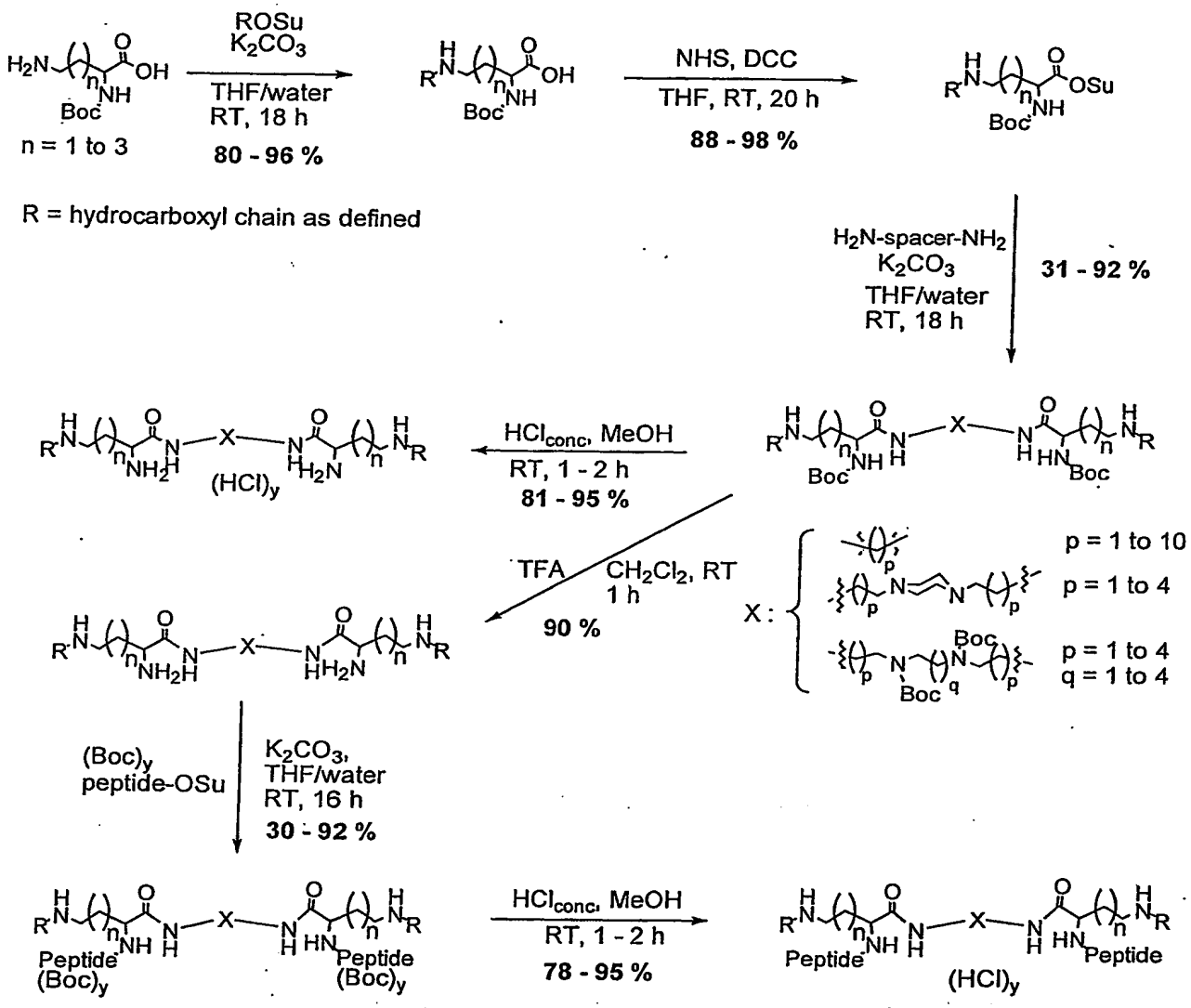

ROSu
K2CO3
THF/water
RT, 18 h
80 - 96 %
n = 1 to 3
NHS, DCC
THF, RT, 20 h
88 - 98 %
R = hydrocarboxyl chain as defined
H2N-spacer-NH2
K2CO3
THF/water
RT, 18 h
31 - 92 %
HClconc, MeOH
RT, 1 - 2 h
81 - 95 %
TFA
CH2Cl2, RT
1 h
90 %
X:
p = 1 to 10
p = 1 to 4
p = 1 to 4
q = 1 to 4
(Boc)y
peptide-OSu
K2CO3,
THF/water
RT, 16 h
30 - 92 %
HClconc, MeOH
RT, 1 - 2 h
78 - 95 %

Figure 2

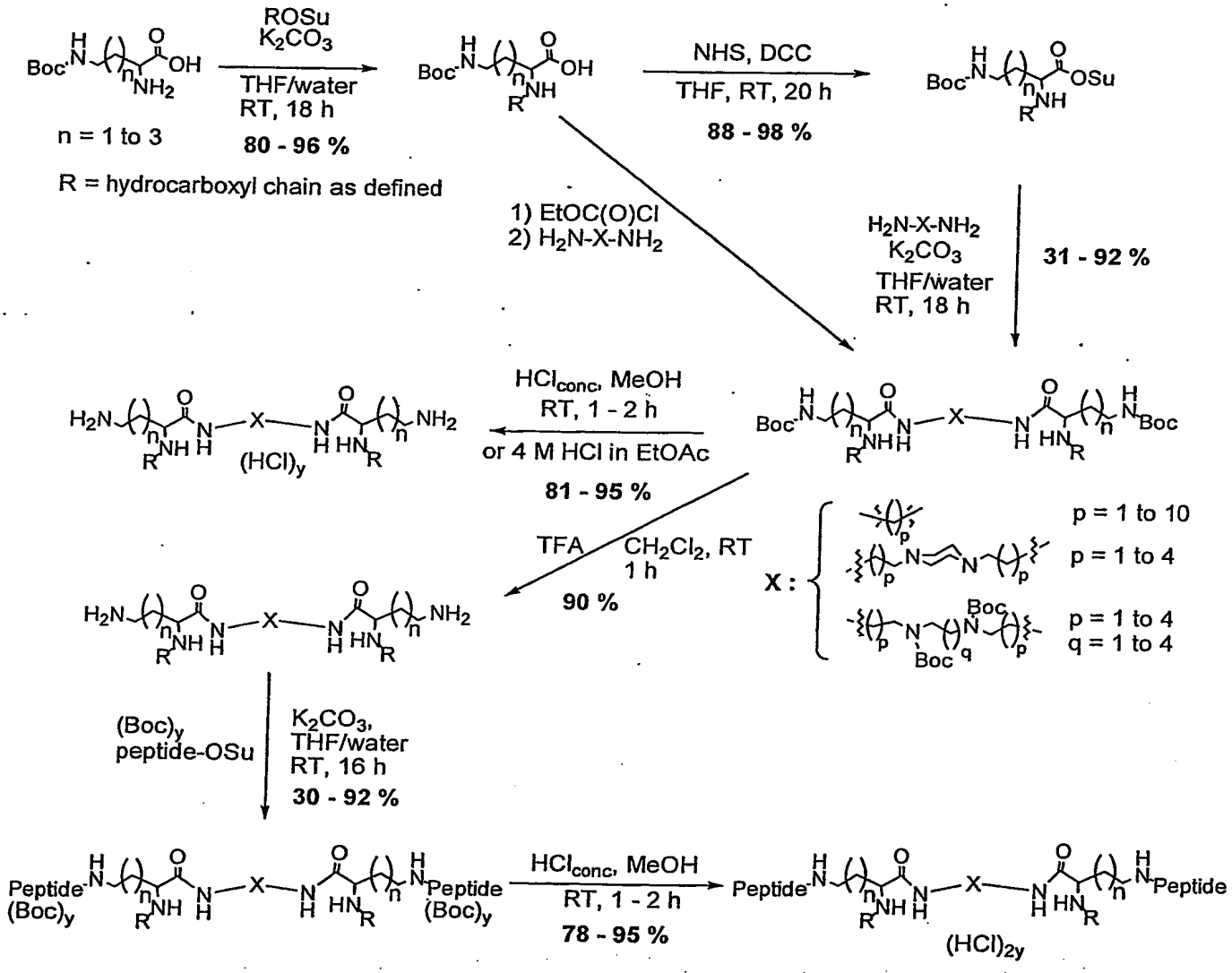

ROSu
K2CO3
THF/water
RT, 18 h
80 - 96 %
n = 1 to 3
R = hydrocarboxyl chain as defined
NHS, DCC
THF, RT, 20 h
88 - 98 %
1) EtOC(O)Cl
2) H2N-X-NH2
H2N-X-NH2
K2CO3
THF/water
RT, 18 h
31 - 92 %
HClconc, MeOH
RT, 1 - 2 h
or 4 M HCl in EtOAc
81 - 95 %
TFA
CH2Cl2, RT
1 h
90 %
X :
p = 1 to 10
p = 1 to 4
p = 1 to 4
q = 1 to 4
(Boc)y
peptide-OSu
K2CO3,
THF/water
RT, 16 h
30 - 92 %
HClconc, MeOH
RT, 1 - 2 h
78 - 95 %

Figure 3

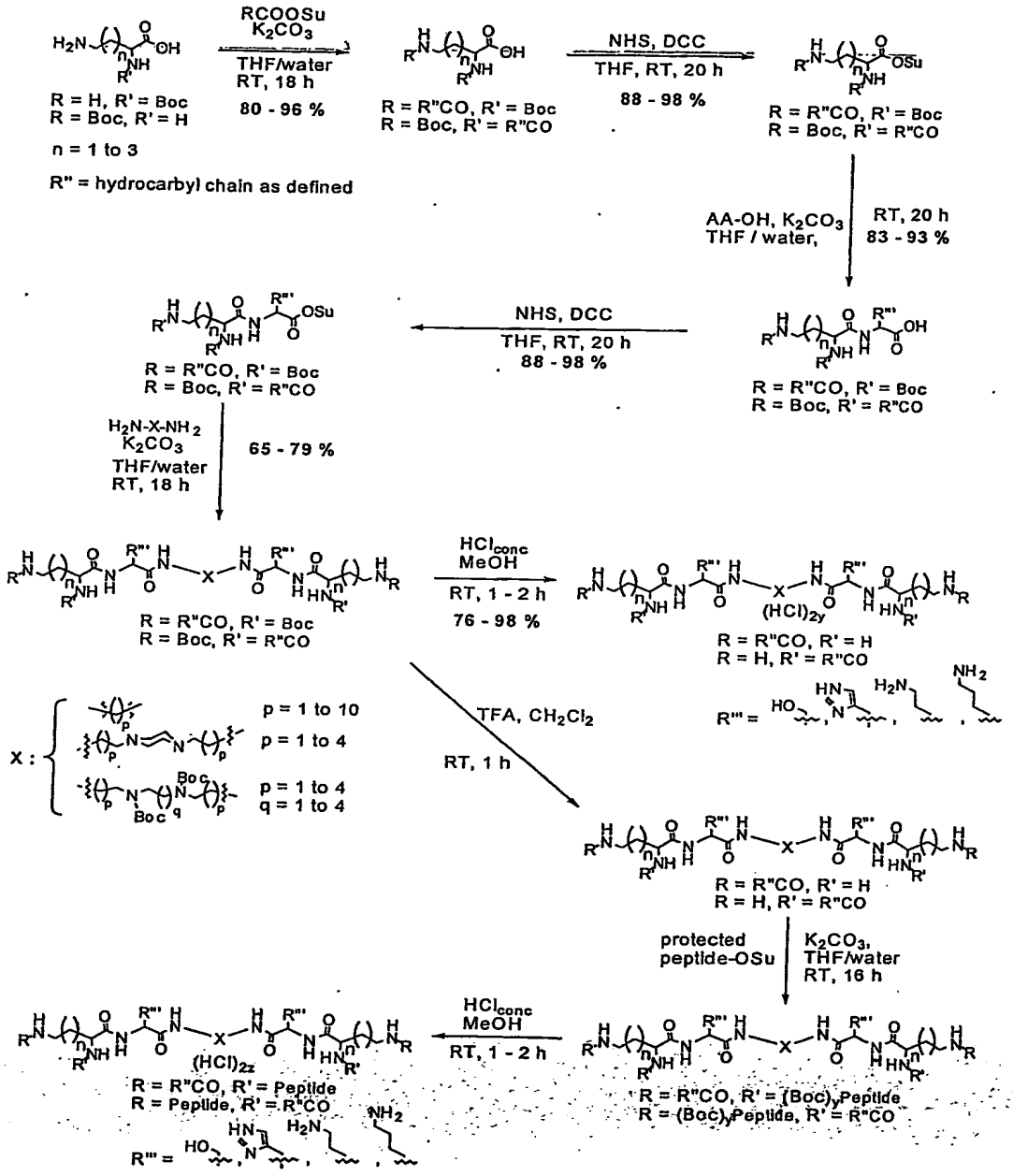

RCOOSu
K2CO3
THF/water
RT, 18 h
80 - 96 %
R = H, R' = Boc
R = Boc, R' = H
n = 1 to 3
R" = hydrocarbyl chain as defined
R = R"CO, R' = Boc
R = Boc, R' = R"CO
NHS, DCC
THF, RT, 20 h
88 - 98 %
AA-OH, K2CO3
THF / water,
RT, 20 h
83 - 93 %
H2N-X-NH2
K2CO3
THF/water
RT, 18 h
65 - 79 %
HClconc
MeOH
RT, 1 - 2 h
76 - 98 %
(HCl)2y
TFA, CH2Cl2
RT, 1 h
p = 1 to 10
p = 1 to 4
p = 1 to 4
q = 1 to 4
protected
peptide-OSu
K2CO3,
THF/water
RT, 16 h
(HCl)2z
R = (Boc)yPeptide
R = Peptide

Figure 4

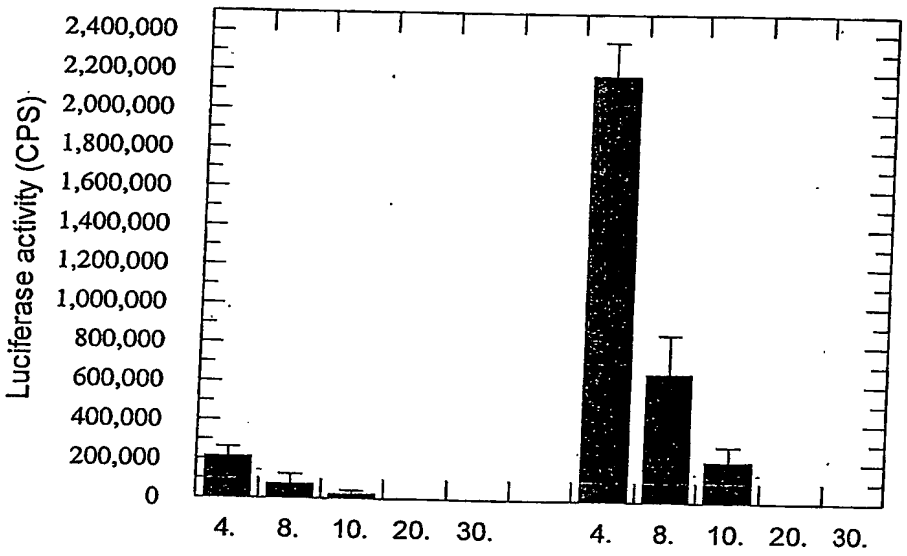

Luciferase activity (CPS)
2,400,000
2,200,000
2,000,000
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
4.
8.
10.
20.
30.
4.
8.
10.
20.
30.

Figure 5

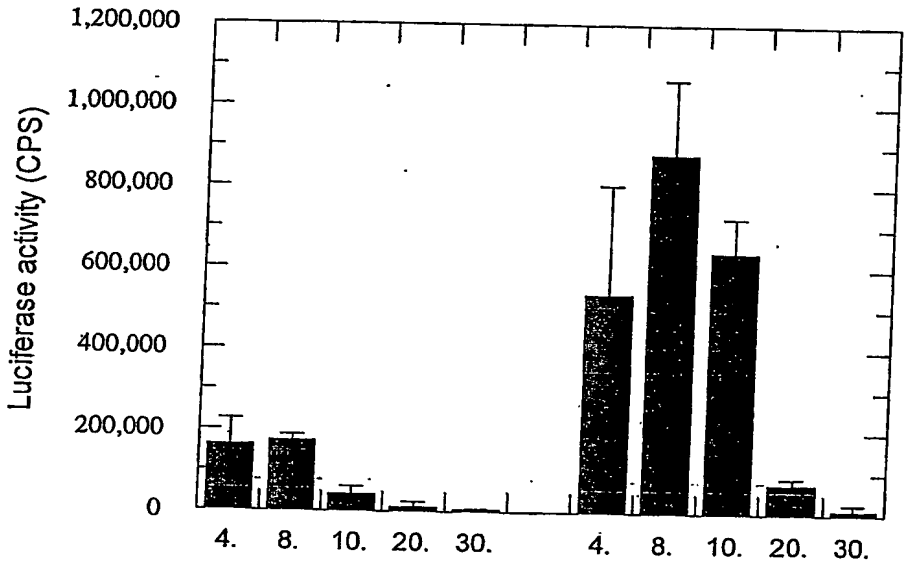
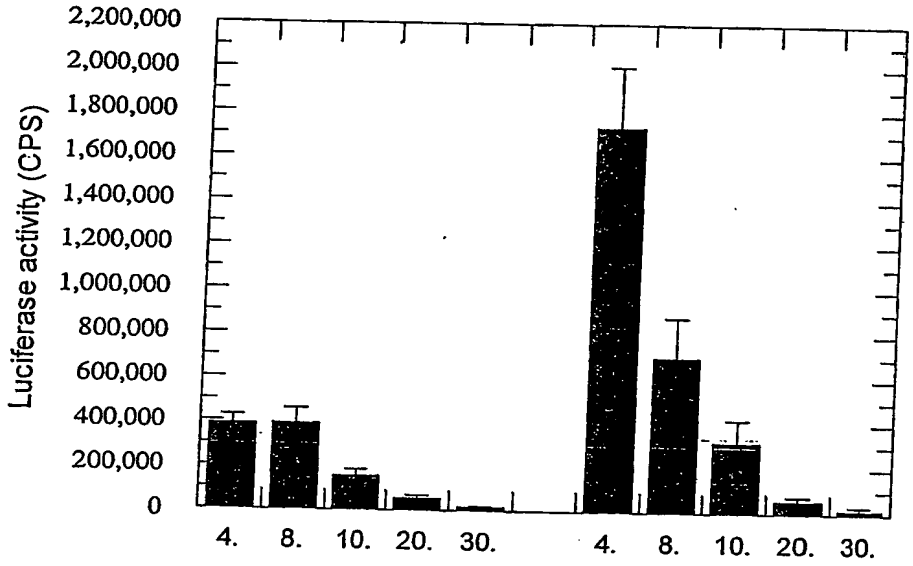

1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
Luciferase activity (CPS)
4.
8.
10.
20.
30.
4.
8.
10.
20.
30.

Figure 6


Luciferase activity (CPS)
2,200,000
2,000,000
1,800,000
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
4.
8.
10.
20.
30.
4.
8.
10.
20.
30.